Pilot studies to develop and evaluate a muscle strengthening programme to reduce the risk of aspiration and improve outcome in stroke patients

Kulnik, Stefan Tino

Awarding institution:
King's College London

PILOT STUDIES TO DEVELOP AND EVALUATE A MUSCLE STRENGTHENING PROGRAMME TO REDUCE THE RISK OF ASPIRATION AND IMPROVE OUTCOME IN STROKE PATIENTS

Stefan Tino KULNIK
PhD Clinical Neuroscience

Abstract

Stroke can impair cough function. Respiratory muscle training (RMT) was investigated as an intervention for improving cough function in acute stroke; and as a potential strategy for preventing aspiration-related post-stroke pneumonia.

Measures of cough function (volitional tests of cough flow and respiratory muscle strength, automated cough frequency measurement) required validation in the acute stroke population. Test-retest reliability was equally high in eleven healthy volunteers and six stroke survivors (ICCs >0.90). Minimal detectable difference was ≈7%. A calibrated pneumotachograph was found most appropriate for cough flow assessments, due to inaccuracy of portable flow meters (Bland-Altman 95% limits of agreement spanning ≈150 L/min). Automated cough frequency measurements (Leicester Cough Monitor) showed high accuracy (ICC >0.99).

The effectiveness of RMT was investigated in a single-blind randomised placebo-controlled trial of 82 acute stroke survivors in three parallel groups (inspiratory, expiratory, and sham training). Mean group changes from baseline (SEM), respectively, were: 91 (42), 49 (27) and 84 (34) L/min for peak voluntary cough flow (p=0.46); -4 (28), 17 (19) and 32 (18) L/min for peak reflex cough flow (p=0.41); 20 (4), 12 (3) and 12 (4) cmH_2O for maximal expiratory mouth pressure (p=0.35); and 18 (4), 10 (3) and 14 (3) cmH_2O for maximal inspiratory mouth pressure (p=0.40). Pneumonia occurred in 13 (16%) participants with no difference between groups (p=0.65). Higher voluntary cough flow at baseline predicted lower pneumonia risk in patients with unsafe swallow (OR 0.73, 95%CI 0.51-0.95, p=0.012), but not in patients with safe swallow. In a sub-group of 21 patients, 24-hour cough frequency was abnormally high at baseline (median (range) 118 (4, 375)) and decreased to 56 (1, 186) at four weeks and 34 (6, 108) at twelve weeks (p=0.0003).

RMT did not improve cough flow or respiratory muscle strength beyond natural recovery. Stronger cough was protective from aspiration-related post-stroke pneumonia.

Publications

KULNIK, S.T., MACBEAN, V., BIRRING, S.S., MOXHAM, J., RAFFERTY, G.F. and KALRA, L., 2015. Accuracy of Portable Devices in Measuring Peak Cough Flow. *Physiological Measurement*, **36**(2), pp. 243-257. [Epub ahead of print]

KULNIK, S.T., BIRRING, S.S., MOXHAM, J., RAFFERTY, G.F. and KALRA, L., 2015. Does Respiratory Muscle Training Improve Cough Flow in Acute Stroke? Pilot Randomized Controlled Trial. *Stroke*, **46**(2), pp. 447-453.

KULNIK, S.T., RAFFERTY, G.F., BIRRING, S.S., MOXHAM, J. and KALRA, L., 2014. A Pilot Study of Respiratory Muscle Training to Improve Cough Effectiveness and Reduce the Incidence of Pneumonia in Acute Stroke: Study Protocol for a Randomized Controlled Trial. *Trials*, **15**: 123.

Table of Contents

Table of Figures

Table of Tables

Acknowledgements

I would like to express my gratitude to all who have supported me during my studies through their advice, guidance, encouragement and example, in particular my supervisors, Lalit Kalra, Surinder Birring and Gerrard Rafferty; and Dimitra Nikoletou, Ines Kralj-Hans, John Hodsoll and John Moxham. I would like to thank my fellow students and researchers for their invaluable peer support: Victoria MacBean, Arietta Spinou, Sarah Chamberlain, Bronwen Connolly, Nyma Sikondari, Alan Lunt, Ndaba Mazibuko, Matthew Maddocks, Ross Laxton, Rok Berlot, Ross Pollock, and many others. I am thankful to Maria Pires for translating one of my references from the Portuguese language. I would also like to thank colleagues in clinical practice, whose support contributed to making my research work that little bit easier; in particular the respiratory physiology team at the King's College Hospital Chest Unit, and the nursing, therapies and medical teams at the King's College Hospital and University Hospital Lewisham stroke units.

I owe my deepest gratitude to the stroke survivors who participated in my studies; and who, during a difficult period in their life, were generous in giving some of their time, energy and focus to this research.

Lastly, I would like to thank my wife and my parents, who have supported me throughout this academic endeavour – I could not have done it without you.

Contributors

The randomised controlled trial of respiratory muscle training in acute stroke was conceived and the study designed by Professor Lalit Kalra. Dr Ross Pollock recruited and collected data for the first 23 participants to the randomised controlled trial, from March to December 2011. Dr John Hodsoll advised on the statistical analyses of trial data, in his role with the Statistics Advisory Service at the Institute of Psychiatry, Psychology and Neuroscience.

Dr Surinder Birring supervised the study of cough frequency in acute stroke and supplied Leicester Cough Monitor (LCM) devices. Dr Gerrard Rafferty supervised the study of portable devices for cough flow measurement and provided access to equipment and facilities at the Respiratory Muscle Laboratory at King's College Hospital.

Abbreviations

ACE	Angiotensin-Converting Enzyme
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
ATPS	Ambient Temperature, Pressure, Water Vapour Saturated
ATS	American Thoracic Society
AUC	Area Under the Curve
bpm	Beats Per Minute
BRT	Breathing Retraining Exercises
BSA	Bedside Swallow Assessment
BTPS	Body Temperature, Pressure, Water Vapour Saturated
ºC	Degrees Celsius
CI	Confidence Interval
cm	Centimetre
cmH_2O	Centimetres of Water
CO_2	Carbon Dioxide
Coef	Coefficient
CONSORT	Consolidated Standards of Reporting Trials
COPD	Chronic Obstructive Pulmonary Disease
CVE	Cough Volume Expired
CVAC	Cough Volume Acceleration
CVI	Cough Volume Inspired
DRG	Dorsal Respiratory Group
EMT	Expiratory Muscle Training
ERS	European Respiratory Society
ERV	Expiratory Reserve Volume
ES	Effect Size
EU	European Union
FEF_{25-75}	Forced Expiratory Flow Between 25% and 75% of the Expired Volume
FEV_1	Forced Expiratory Volume in One Second

FRC	Functional Residual Capacity
FVC	Forced Vital Capacity
GBP	Pound Sterling
GCT	Glottis Compression Time
H_2O	Water
HRpeak	Peak Heart Rate
Hz	Hertz
IC	Inspiratory Capacity
ICC	Intraclass Correlation Coefficient
ID	Internal Diameter
IMT	Inspiratory Muscle Training
IQR	Interquartile Range
IPPB	Intermittent Positive Pressure Breathing
IRV	Inspiratory Reserve Volume
ISRCTN	International Standard Randomised Controlled Trial Number
L	Litre
L/min	Litres per Minute
L/s/s	Litres per Second per Second
LCM	Leicester Cough Monitor
ln	Natural Logarithm
logSD	Standard Deviation of the Logarithmically Transformed Data
µMol/L	Micromoles per Litre
MAR	Missing at Random
mm	Millimetre
mmH_2O	Millimetres of Water
mmHg	Millimetres of Mercury
mL	Millilitre
MVV	Maximal Voluntary Ventilation
NBM	Nil By Mouth
NEADL	Nottingham Extended Activities of Daily Living Questionnaire
NIHSS	National Institutes of Health Stroke Scale

NHS	National Health Service
NRES	National Research Ethics Service
O_2	Oxygen
OR	Odds Ratio
pCO_2	Partial Pressure of Carbon Dioxide
PECF	Peak Expiratory Cough Flow
PEF	Peak Expiratory Flow
PEmax	Maximal Expiratory Mouth Pressure
PEP	Positive Expiratory Pressure
pH	Power of Hydrogen
PICF	Peak Inspiratory Cough Flow
PImax	Maximal Inspiratory Mouth Pressure
pO_2	Partial Pressure of Oxygen
PRG	Pontine Respiratory Group
PSP	Post-Stroke Pneumonia
r^2	Square of the Pearson Correlation Coefficient
r_s	Spearman's Rank Correlation Coefficient
RMT	Respiratory Muscle Training
ROC	Receiver Operated Characteristics
RR	Relative Risk
RV	Residual Volume
SaO_2	Arterial Oxygen Saturation
SD	Standard Deviation
SE	Standard Error
sec	Seconds
SEM	Standard Error of the Mean
SF-36	Short Form 36
tDCS	Transcranial Direct Current Stimulation
TLC	Total Lung Capacity
Tmax	Time of Maximum
TMS	Transcranial Magnetic Stimulation

UK	United Kingdom
USA	United States of America
USD	United States Dollar
VAT	Value Added Tax
VC	Vital Capacity
VD/VTpeak	Peak Dead Space to Tidal Volume Ratio
$V_E$peak	Peak Minute Ventilation
VO_2peak	Peak Oxygen Consumption
VRG	Ventral Respiratory Group
VT	Tidal Volume
W	Watt

Chapter 1 Introduction

The present studies were conducted to investigate respiratory muscle training (RMT) as a means of improving cough effectiveness in adults after acute stroke, with the aim of reducing the risk of pneumonia posed by swallowing difficulty and aspiration in this patient group.

In this introduction, the scene shall be set by first giving an overview of the impact and burden of stroke in today's society in the United Kingdom (UK) (section 1.1). Then follows a narrative review of two key topics, which form the basis of the argument for investigating RMT in acute stroke: pneumonia in stroke (section 1.2) and the impairment of cough in stroke (section 1.3). A description of RMT as a training technique is then given (section 1.4). This introduction is concluded by a systematic review of previous studies of RMT in stroke (section 1.5).

The argument underlying the present research is that pneumonia is a relevant medical complication after stroke, and that the risk of post-stroke pneumonia (PSP) is increased due to swallowing difficulty and aspiration after stroke. Recent studies have shown that cough, the most immediate mechanism protecting the lungs from inhaled foreign materials, can be impaired in stroke survivors; and that the likely cause for this impairment is central weakness of the respiratory musculature. Thus, respiratory muscle training as a treatment intervention in acute stroke may be useful in the recovery of respiratory muscle and cough function, and may reduce the risk of pneumonia.

1.1 Stroke in the United Kingdom today

Stroke, or cerebrovascular accident, is defined as the sudden onset of a focal neurological deficit, due to a local disturbance of blood supply to the brain and the subsequent destruction of brain cells (World Health Organisation 1971). Most commonly, stroke occurs due to lack of blood supply to the brain, caused by narrowing or blockage of cerebral blood vessels (Great Britain. Department of Health 2007). This is termed ischemic stroke and accounts for approximately 85% of all strokes (Intercollegiate Stroke Working Party 2012). The second-most common mechanism of stroke is through bleeding originating in the brain parenchyma (Great Britain. Department of Health 2007). This is termed haemorrhagic stroke and causes approximately 10% of all strokes (Intercollegiate Stroke Working Party 2012).

Stroke is a leading cause of death. In the UK in 2010, stroke caused approximately 7% of all deaths in men and 10% of deaths in women (Townsend *et al*. 2012). Overall, stroke caused almost 50,000 deaths in the UK in 2010, the fourth largest cause of death after cancer, heart disease and respiratory disease (Townsend *et al*. 2012). Data from 2006 show that 17% to 25% of those suffering a stroke die within 60 days of the event (Townsend *et al*. 2012).

Estimates from 2007 and 2009 put the annual incidence of stroke in the UK at approximately 152,000 (Townsend *et al*. 2012). In England in 2007, the annual incidence rate of stroke per 100,000 was 139 for women and 178 for men (Townsend *et al*. 2012). The prevalence of stroke in the UK in 2010 and 2011 is estimated at 1.1 million or 1.8% of the population (Townsend *et al*. 2012). Time-trend studies suggest that in the UK incidence and mortality rates for stroke have decreased over the past decade, while the prevalence of stroke has risen (Lee *et al*. 2011, Bhatnagar *et al*. 2010).

Stroke is a major cause of adult disability (Adamson *et al*. 2004). Audit data for England, Wales and Northern Ireland from 2010 show that at the time of discharge from hospital approximately

42% of stroke survivors have returned to independence, whereas 36% have mild to moderate disability, and 22% have severe or very severe disability (Intercollegiate Stroke Working Party 2011). Stroke has a greater disability impact than other chronic diseases; it causes a greater range of disabilities than any other condition; and it is the largest cause of complex disability in adults in the UK (Adamson *et al*. 2004).

Stroke generates considerable costs to the UK health and social care system and to society in general. Based on data from 2008 and 2009, it is estimated that the annual costs of stroke to services within the health and social care system amount to at least £3 billion. The wider economic costs to society, including benefit payments and lost economic productivity, are estimated at about £8 billion (National Audit Office 2010).

In the past decade, remarkable advancements in health care have led to decreased stroke incidence and mortality rates and improved outcomes after stroke rehabilitation. Major advances in stroke care have been: the introduction of specialised stroke units; thrombolysis within three hours of acute ischemic stroke; aspirin within 48 hours of acute ischemic stroke; and a number of primary and secondary prevention strategies (Donnan *et al*. 2008). Nevertheless, there are many opportunities for further improvement in stroke treatment, prevention and rehabilitation, one of which concerns pneumonia after stroke (Kalra 2010, Donnan *et al*. 2008).

1.2 Post-stroke pneumonia

1.2.1 Incidence of post-stroke pneumonia

Pneumonia is frequently described as a relevant medical complication after stroke. However, an estimation of its incidence and clinical impact is complicated by variations between studies. Studies differ in the criteria for the diagnosis of pneumonia; study settings; characteristics and size of study samples; and time periods of observation for the incidence of pneumonia. Forty-two studies reporting the incidence of pneumonia after stroke were reviewed and are summarised in Appendix 1. These publications span from 1987 to 2012 and present data from intensive care, acute, and rehabilitation settings. Most studies are prospective or retrospective longitudinal observational studies with samples of consecutively admitted stroke patients, thereby providing a picture of the 'naturally' occurring incidence of post-stroke pneumonia (PSP). In some studies additional selection criteria were applied, such as presence of swallowing difficulty, and these are likely to have influenced the observed incidence rates.

The incidence of PSP across all studies ranges from 2% to 57%, with a median incidence rate of 10% and an inter-quartile range from 6.4% to 16.2%. The highest incidence of PSP is reported in intensive care settings, where it ranges from 21% to 57% (Yeh *et al*. 2011, Walter *et al*. 2007, Upadya *et al*. 2004, Hilker *et al*. 2003), reflecting the general vulnerability to respiratory infection of patients requiring intensive care management and mechanical ventilation (Hoffmann *et al*. 2012). The most current PSP rates for the UK are derived from the Royal College of Physicians bi-annual stroke audit for England, Wales and Northern Ireland. The audit captures consecutive admissions to acute stroke services for the period from April to June. In 2008, 16% of acute stroke patients included in the audit developed pneumonia during their hospital stay, compared with 13% in 2010 (Royal College of Physicians 2011, 2009).

While most studies focus on pneumonia during the first weeks after stroke, mainly for the duration of the patient's acute hospital admission or rehabilitation stay, some studies have

observed PSP incidence over longer time periods. Sellars *et al*. (2007) reported confirmed pneumonia during the first three months after stroke in 18.9% and suspected pneumonia in 19.9% of patients. Masiero *et al*. (2008) reported an incidence of pneumonia within the first six months after stroke of 13.4%. In the study by Mann *et al*. (1999), 9% of patients developed pneumonia in the first week after stroke, 12% within the first month, and 20% within six month of stroke. Langhorne *et al*. (2000) observed the incidence of pneumonia for 30 months following stroke and reported an incidence rate of 22% from hospital admission to two months; 13% from discharge from hospital to six months; 23% from six to 18 months; and 29% from 18 to 30 months. These limited long-term data indicate that PSP may be as relevant in chronic stroke as in the acute phase of stroke.

In summary, while incidence rates for PSP are widely reported in the international literature, these figures should be appreciated taking into account varying definitions of pneumonia and other differences between studies. As a general estimate, the incidence of pneumonia between stroke onset and hospital discharge lies between approximately 6% and 16% internationally. Rates are likely to be most valid for the particular setting and time period, and it may not be appropriate to compare directly between studies. The most current and valid data for the UK is probably from the national stroke audit for England, Wales and Northern Ireland, which showed incidence rates of 16% in 2008 and 13% in 2010.

1.2.2 Post-stroke pneumonia and stroke outcomes

Several studies investigated the association of PSP with mortality, disability, length of hospital stay and cost of care after stroke. Authors generally describe that PSP is associated with increased risk of death, poorer rehabilitation outcomes, increased length of hospital stay and increased healthcare costs, although the reported magnitude of these risks and negative implications varies. As with incidence rates of PSP, it may be most appropriate to appreciate reported figures in the context of the individual studies' sample characteristics, health care settings, time periods, and data collection and analysis methods.

Several studies analysed the association between pneumonia and mortality after stroke (Wilson 2012, Finlayson *et al*. 2011, Koennecke *et al*. 2011, Tong *et al*. 2010, Saposnik *et al*. 2008, Sellars *et al*. 2007, Ovbiagele *et al*. 2006, Hinchey *et al*. 2005, Aslanyan *et al*. 2004, Heuschmann *et al*. 2004, Katzan *et al*. 2003, Vernino *et al*. 2003), reporting adjusted odds ratios in the range from 1.9 (Saposnik *et al*. 2008) to 6.6 (Heuschmann *et al*. 2004). Studies reporting the causes of death after acute stroke (Hong *et al*. 2008, Vernino *et al*. 2003, Henon *et al*. 1995, Derouesne *et al*. 1993, Oppenheimer & Hatchinski 1992, Viitanen *et al*. 1987) further link PSP with increased mortality, with the cause of death being attributed to pneumonia in up to 35% of stroke deaths (Oppenheimer & Hatchinski 1992). These studies also show that within the first week after stroke, death is most frequently attributed to neurological causes, mostly increased intracranial pressure. Whereas in the following weeks, medical complications, including pneumonia, become more pertinent (Derouesne *et al*. 1993, Oppenheimer & Hachinski 1992, Viitanen *et al*. 1987, Silver *et al*. 1984, Brown 1973). One study reported a relative risk of death of 4.9 for patients who developed PSP over a ten-year period compared with those who did not (Vernino *et al*. 2003).

Several studies demonstrate that post stroke pneumonia is associated with poorer rehabilitation outcomes, increased level of disability and increased use and cost of acute health care resources. Ovbiagele *et al*. (2006) reported that patients with PSP were less likely to walk without assistance at the time of discharge compared with those without pneumonia, while other studies reported that pneumonia was associated with worse National Institutes of Health Stroke Scale (NIHSS) score, worse Barthel Index, and worse Rankin Scale score (Finlayson *et al*. 2011, Koennecke *et al*. 2011, Hong *et al*. 2008, Aslanyan *et al*. 2004). Increased length of hospital stay for stroke patients with pneumonia was reported by Finlayson *et al*. (2011), Tong *et al*. (2010), Ovbiagele *et al*. (2006) and Hinchey *et al*. (2005). In the study by Katzan *et al*. (2007), patients who developed pneumonia were more likely to require extended care after discharge from the acute hospital, and were more likely to be re-admitted to the acute hospital within 30 days of discharge. In the United States of America (USA), the additional hospitalisation cost related to PSP was estimated at USD 15,000 (Katzan *et al*. 2007) and USD 27,366 (Wilson 2012).

In summary, patients who develop pneumonia after stroke are more likely to die than those who do not, with an estimated two- to six-fold increase in risk of death. This estimate is derived mainly from data pertaining to the first weeks and months after stroke, although one long-term study matches this estimate with a suggested five-fold increase in risk of death over ten years. Some evidence is available to show that patients with post-stroke pneumonia have worse rehabilitation outcomes within the first weeks and months after stroke, as they are more likely to have poor scores on various rehabilitation indicators compared with those who did not develop pneumonia. Some studies indicate the potential additional cost of PSP to health care providers, as on average patients with pneumonia stay in the acute hospital for longer than those without pneumonia, and also require higher levels of care after hospital discharge.

1.2.3 Risk factors for post-stroke pneumonia

A number of studies have described risk factors for pneumonia after stroke and are listed in Table 1. Studies conducted in intensive care settings have been omitted here, since ventilator-associated pneumonia may be regarded as a separate clinical presentation (Hoffmann *et al*. 2012). To identify risk factors for PSP, most authors applied multivariate regression methods and adjusted for participant characteristics, whereby the number of variables accounted for differs between studies. Most commonly, risk factor analyses were adjusted for patients' age, sex, stroke severity at admission and presence of dysphagia. The studies differ with respect to sample characteristics, sample size, clinical setting and details of statistical analysis methods. An appreciation of the results in the particular context of individual studies is therefore advisable. The identification of risk factors in these studies is also influenced by the *a priori* selection of potential candidate variables and the availability of data.

Table 1. Risk factors for post-stroke pneumonia reported in the literature

Risk factor	Studies	Range of adjusted odds ratios (95% CI)
Higher level of stroke impairment	Hoffmann *et al*. 2012 Shaheen *et al*. 2012 Finlayson *et al*. 2011 Chumbler *et al*. 2010 Royal College of Physicians 2009 Indredavik *et al*. 2008 Sellars *et al*. 2007 Hinchey *et al*. 2005 Aslanyan *et al*. 2004 Roth *et al*. 2001	1.1 (1.0, 1.1) (Aslanyan *et al*. 2004) to 14.7 (7.0, 25.6) (Sellars *et al*. 2007)
Presence of swallowing impairment	Hoffmann *et al*. 2012 Shaheen *et al*. 2012 Finlayson *et al*. 2011 Chumbler *et al*. 2010 Lakshminarayan *et al*. 2010 Royal College of Physicians 2009 Sellars *et al*. 2007 Martino *et al*. 2005 Mann *et al*. 1999 Smithard *et al*. 1996	1.9 (1.5, 2.4) (Finlayson *et al*. 2011) to 11.6 (3.4, 39.8) (Martino *et al*. 2005)
Older age	Aslanyan *et al*. 2004 Royal College of Physicians 2009 Chumbler *et al*. 2010 Hoffmann *et al*. 2012 Finlayson *et al*. 2011 Ovbiagele *et al*. 2006 Sellars *et al*. 2007	1.1 (1.0, 1.1) (Aslanyan *et al*. 2004) to 3.9 (2.0, 7.5) (Sellars 2007)
Male sex	Aslanyan *et al*. 2004 Finlayson *et al*. 2011 Hoffmann *et al*. 2012 Katzan *et al*. 2003	1.7 (1.4, 2.0) (Hoffmann *et al*. 2012) to 1.9 (1.6, 2.3) (Finlayson *et al*. 2011)
Reduced level of consciousness	Dziewas *et al*. 2004 Katzan *et al*. 2003 Masiero *et al*. 2008 Royal College of Physicians 2009	5.6 (2.2, 16.0) (Masiero *et al*. 2008) to 7.4 (2.9, 18.4) (Dziewas *et al*. 2004)

The most frequently reported risk factors for PSP are increasing stroke severity, older age, the presence of swallowing difficulty, male sex and reduced level of consciousness. In the context of the present research, the interaction between aspiration, cough and PSP is of particular interest. Synthesising the evidence on dysphagia as a risk factor for PSP, Martino *et al*. (2005) conducted a systematic review and meta-analysis, which included nine studies (Lim *et al*. 2001, Mann *et al*. 1999, Sala *et al*. 1998, Chua & Kong 1996, Gottlieb *et al*. 1996, Smithard *et al*. 1996, Kidd *et al*. 1995, Holas *et al*. 1994, Gordon *et al*. 1987). Using broad criteria for the detection of dysphagia, from 'soft' clinical signs to findings from instrumental swallow assessments, these studies combined gave a sample of 400 dysphagic patients, out of whom 87 developed PSP, and 491 non-dysphagic patients, out of whom 29 developed PSP. The combined relative risk (95% CI) for developing pneumonia in the dysphagic group was 3.2 (2.1, 4.9). A meta-analysis of studies reporting the incidence of aspiration and pneumonia after stroke included 51 patients who aspirated, out of whom 22 developed PSP, and 59 non-aspirators, out of whom 2 developed PSP. The combined relative risk (95% CI) for developing pneumonia for those with aspiration was 11.6 (3.4, 39.8) (Martino *et al*. 2005).

Further aspects of dysphagia and aspiration in relation to the risk of PSP have been reported. In the study by Holas *et al*. (1994), the risk of developing pneumonia was higher for those who aspirated silently (*i.e.* aspirated without eliciting a protective cough) compared with those who coughed on aspiration or did not aspirate (RR 5.6, 95% CI 1.4, 21.3); and higher for those who aspirated 10% or more of the swallowed material compared with those who aspirated less than 10% or did not aspirate (RR 8.4, 95% CI 2.7, 25.5). In the study by Johnson *et al*. (1993), longer pharyngeal transit time (time for the bolus to pass through the pharynx, and for the epiglottis to return to its original position) was associated with an increased risk of PSP. Vallecular pooling, piriform pooling and penetration to or through the true vocal cords were also associated with an increased incidence of PSP, although this did not reach statistical significance (Johnson *et al*. 1993). In the study by Masiero *et al*. (2008), the lack of reflex cough after swallow (indicating silent aspiration) was associated with an increased risk of pneumonia (adjusted OR 7.6, 95% CI 1.8, 30.0). The studies by Addington *et al*. (1999a, 2005) showed that a reduced or absent cough reflex was associated with increased PSP incidence, highlighting the risk posed by silent aspiration and the protective function of cough from aspiration pneumonia. Overall, these

studies demonstrate that dysphagia in general is associated with an approximately two- to three-fold increase in risk of pneumonia after stroke. This risk increases to five- to eleven-fold with increasing severity of swallowing difficulty, the presence of aspiration, and increasing severity of aspiration.

In summary, studies have identified a variety of risk factors for PSP. The most consistently reported predictors are increasing stroke severity, older age, the presence of swallowing difficulty, male sex and reduced level of consciousness. The reported magnitude of these risks can vary considerably, and it may be advisable to appreciate figures in the context of the particular study characteristics. A number of studies demonstrate the link between aspiration, impaired cough and PSP. This evidence lends support to the rationale for the present research, which aimed to reduce pneumonia by increasing cough effectiveness and improving airway protection from aspiration.

1.2.4 Strategies for the prevention of post-stroke pneumonia

To date, the most widely used strategy for the prevention of PSP is the routine screening of stroke patients for swallowing difficulty coupled with the implementation of dysphagia management strategies. This approach is based on the established association between dysphagia and pneumonia incidence. Accordingly, national clinical guidelines for stroke recommend screening for dysphagia prior to allowing the patient to eat and drink (Intercollegiate Stroke Working Party 2012, National Collaborating Centre for Chronic Conditions 2008, Great Britain. Department of Health 2007). The studies by Ickenstein *et al*. (2010), Lakshminarayan *et al*. (2010) and Hinchey *et al*. (2005) provide evidence for the effectiveness of formalised routine dysphagia screens for the prevention of PSP. Hinchey *et al*. (2005) compared stroke units which operated with either a formal or informal swallow screen. Units with a formal swallow screen had higher adherence to swallow screening (78% compared with 56%) and lower incidence of PSP (2.4% compared with 5.3%). In the study by Lakshminarayan *et al*. (2010) the adjusted odds ratio for developing pneumonia for those who failed dysphagia screening, compared with those who passed dysphagia screening, was 3.6 (95% CI 3.0, 4.3). In addition,

those patients who did not undergo a swallow screen also had an increased risk of pneumonia compared with those who passed swallow screening, with an adjusted odds ratio of 2.2 (95% CI 1.7, 2.7). In the study by Ickenstein *et al*. (2010), the annual incidence of pneumonia for patients admitted to one stroke unit dropped from 7.4% to 2.8% after the introduction of a structured swallowing screen.

There is some evidence to indicate that the introduction of specialised stroke units also reduces the incidence of PSP. In the study by Kalra *et al*. (1995), patients who required further hospital care two weeks after stroke were randomised to a general ward or a stroke unit setting. The incidence of pneumonia was 8% for stroke unit patients, compared with 16% for general ward patients. In the study by Ickenstein *et al*. (2010) the in-house incidence rate for PSP dropped from 12.4% to 7.4% following the introduction of a dedicated stroke unit. The mechanism for this is unclear and may be related to heightened alertness of staff towards the possibility of dysphagia and aspiration pneumonia in acute stroke patients. Also, a concerted multidisciplinary patient management approach may contribute to reducing the risk of PSP. Optimised patient positioning and early mobilisation may positively affect respiratory function (Lee *et al*. 2012), and adequate positioning, assistance and supervision provided during meal times may facilitate swallowing function and reduce the risk of aspiration.

When investigating an intervention to reduce PSP risk it may seem intuitive to focus on those stroke patients who are identified as having swallowing difficulty, due to the evidence for dysphagia and aspiration as risk factors for PSP. However, while swallowing difficulty and aspiration increase the risk of PSP, it is the case that not all stroke patients with swallowing problems develop pneumonia; and that stroke patients who are not identified clinically as having swallowing problems may also develop pneumonia. The picture of predictors and circumstances of PSP is multi-factorial. While clinically detected dysphagia plays a key part in this picture, it has to be considered that patients who have passed a cursory swallow screen and are deemed to have a ‘safe’ swallow may still have swallowing difficulty. Some evidence for this is provided by the reported frequency of dysphagia after stroke, which is generally lowest according to results of swallow screening, higher with specialist clinical bedside assessment, and highest

with instrumental swallow assessment (Martino *et al*. 2005). This indicates that more detailed assessments of swallow are likely to identify more instances of swallowing difficulty. It is therefore possible that a portion of those stroke patients who pass a cursory swallow screen have some degree of undetected dysphagia or aspirate silently. Furthermore, several authors suggest that aspiration pneumonia should be considered as a possibility without any relationship to the swallowing of food and drink (Teramoto 2009). It is suggested that the aspiration of nasal, throat and periodontal secretions, which mainly occurs overnight, should be regarded as a separate form of silent aspiration, with its own association to PSP (Teramoto 2009). For these reasons, the present studies recruited stroke patients with and without clinically detected swallowing difficulty.

Despite the success of screening for swallowing difficulty and dysphagia management strategies in preventing PSP, there remains scope for further developments to reduce pneumonia rates after stroke. Various avenues have been suggested and researched, based on different patho-physiological and clinical justifications. Pharmacological approaches include the preventive administration of antibiotics to reduce fever and infection (Westendorp *et al*. 2012), the use of angiotensin-converting enzyme (ACE) inhibitors to improve reflex cough sensitivity (Shinohara & Origasa 2012), selective decontamination of the digestive tract to minimise exposure to pathogens (Gosney *et al*. 2006), and pharmacological agents targeting stroke-induced immuno-suppression (Braun *et al*. 2007, Prass *et al*. 2003). Non-pharmacological strategies include elevated positioning to prevent aspiration (Teramoto 2009), passive mobilisation and re-positioning regimes to improve lung ventilation and airway clearance (Cuesy *et al*. 2010), and intensive oral hygiene and dental treatment to reduce oro-pharyngeal colonisation with pathogens (Teramoto 2009).

The present research took a novel and original approach in investigating RMT as a non-pharmacological intervention, with the aim to improve cough effectiveness and increase airway protection in acute stroke patients. The following section shall discuss human cough; its impairment after acute stroke; and the rationale for RMT as a method for improving cough effectiveness in this patient group.

1.3 Impairment of cough in stroke

1.3.1 Normal respiratory function

The movement of air in and out of the lungs is termed ventilation. Ventilation is achieved through respiratory muscle action. Respiratory muscles are distinguished into inspiratory and expiratory muscles. Inspiratory muscle contraction causes air to be drawn into the lungs; expiratory muscle action causes air to be forced out of the lungs. The main inspiratory muscle is the diaphragm, which spans across the lower thorax and separates the thoracic and abdominal compartments. The external intercostal muscles also act as inspiratory muscles. The main expiratory muscles are the abdominal muscles and the internal intercostal muscles. In addition, a number of other skeletal muscles can contribute to inspiration or expiration (scaleni, sternocleido-mastoid, descending trapezius, pectoralis major and minor, latissimus dorsi, and other muscles contributing to movement or stability of the chest wall and the thoraco-abdominal unit); these are termed accessory respiratory muscles (Sieck & Gransee 2012, Petersen 2007).

Respiratory mechanics can be likened to a bellows-type system. Contraction of the diaphragm creates negative pressure in the respiratory tract, which results in inspiratory airflow. When the diaphragm relaxes, passive recoil of the lungs and chest wall generates expiratory airflow. Thus, at rest inspiration is an active process, while expiration is passive. At higher respiratory demands, expiratory muscle contraction causes forced expiration, thereby shortening expiration time and contributing to an increased rate of breathing. Accessory respiratory muscles are usually only activated at high respiratory demands, for example during exercise or in respiratory disease (Sieck & Gransee 2012, Petersen 2007).

The total volume of air occupying the lungs after a maximal inspiratory effort (approximately 6 L) is termed total lung capacity (TLC). The lungs cannot be entirely emptied, and after a maximal expiration there remains the residual volume (RV) of approximately 1.2 L. The volume of air that can actively be shifted in and out of the lungs is termed vital capacity (VC), and is in effect TLC

less RV. The volume of air moved in and out of the lungs during relaxed breathing is termed tidal volume (VT). In healthy adults, the tidal volume is about 500 mL at rest. The volume that remains in the lungs at the end of a relaxed expiration is the functional residual capacity (FRC). FRC is approximately 2.5 L. With each breath, it is replenished with O_2 and CO_2 is removed. The volume that can be inspired with a maximal inspiratory effort after a relaxed expiration is termed the inspiratory capacity (IC). The volume that can be expired with a prolonged expiratory effort after a relaxed expiration is termed the expiratory reserve volume (ERV). The volume that can be inspired with a prolonged inspiratory effort after a relaxed breath in is termed inspiratory reserve volume (IRV) (Petersen 2007, Despopoulos & Silbernagel 2003).

The neural control of respiration is arranged in a complex network involving the brainstem (medulla oblongata and pons), as well as higher brain centres (hypothalamus, limbic system, and cerebral cortex). The continuous rhythm of breathing is generated in the brainstem centres and modulated through sensory afferents according to metabolic demands. Higher centres influence breathing through stimuli such as pain, temperature or emotion; and through voluntary control, for example in breath-holding or other voluntary respiratory manoeuvres (Petersen 2007, Despopoulos & Silbernagel 2003).

The brainstem network has pontine and medullary components. The pontine respiratory group (PRG) includes expiratory neurons in the nucleus parabrachialis medialis, and inspiratory neurons in the nucleus parabrachialis lateralis and Kölliker-Fuse nucleus. The medullary areas are the dorsal respiratory group (DRG), ventral respiratory group (VRG) and Bötzinger complex. The DRG, part of the nucleus of the tractus solitarius, consists mainly of inspiratory upper motor neurons, which project ipsilaterally to the lower motor neurons of mainly the phrenic nerve. They also show inhibitory action on the expiratory motor neurons of the VRG. The VRG is located rostrally in the nucleus ambiguous and caudally in the nucleus retroambigualis. The nucleus ambiguous contains inspiratory upper motor neurons as well as motor neurons to the laryngeal muscles and parasympathetic neurons to the bronchioles and heart. The rostral nucleus retroambigualis contains inspiratory upper motor neurons. The inspiratory neurons in the VRG mainly supply the external intercostals and the accessory muscles, as opposed to the

diaphragm. The caudal nucleus retroambigualis contains expiratory upper motor neurons supplying the internal intercostals and abdominals. During relaxed breathing, these expiratory neurons do not cause discharge of the corresponding lower motor neurons. They inhibit inspiratory motor neurons in the late inspiration, and contribute to the termination of inspiration. The Bötzinger complex is located rostral to the nucleus ambiguous and contains expiratory neurons, which inhibit inspiratory neurons in the DRG and VRG and excite expiratory neurons in the VRG. The exact mechanism of respiratory rhythm generation is not clear. The neurons in the pre-Bötzinger complex, located rostral to the Bötzinger complex, display a cyclical firing pattern, which may act as a respiratory pacemaker. An alternative hypothesis considers a neural network of local re-excitation with reciprocal inhibition of inspiratory and expiratory neural pools (Petersen 2007).

The higher brain centres influence respiration via various pathways. The DRG receives input from the cerebral cortex, limbic system, hypothalamus and cerebellum in response to temperature, pain, emotional stimuli and exercise. In contrast, all voluntary control of respiration (taking deep breaths, performing forced expirations, hyperventilating deliberately, holding one's breath, etc) are initiated from the cerebral cortex, bypassing the brainstem centres of respiration and traveling through the pyramidal system directly to the lower motor neurons (Petersen 2007).

Respiration is modulated by afferent input from chemoreceptors and mechanoreceptors via the nucleus of the tractus solitarius in the medulla oblongata. The principal control of ventilation occurs through the monitoring of arterial pO_2, pCO_2 and pH in the peripheral and central chemoreceptors. The peripheral chemoreceptors are the carotid bodies, located at the bifurcations of the internal and external carotid arteries and connecting to sensory fibres of the glossopharyngeal nerve; and the aortic bodies, which are scattered over the aortic arch and the main arteries in that area, and which connect to afferent fibres of the vagus nerve. The central chemoreceptors are located at the ventrolateral surface of the medulla oblongata. The pattern of breathing (respiratory rate and depth of breathing) is adjusted in order to maintain the blood acid-base balance through the carbonic-acid bicarbonate buffer system. Through this buffer system, excess hydrogen ions are removed from the blood as they are bound in H_2O, while CO_2

is removed from the blood through the lungs. Breathing is more sensitive to stimulation by increased pCO_2 (hypercapnia) than decreased pO_2 (hypoxia). Thereby, ventilation is matched to metabolism through the CO_2 produced rather than the O_2 consumed (Petersen 2007).

Several mechanoreceptors influence the pattern of breathing. Lung-stretch receptors are situated in the smooth muscle of the trachea and lower airways and connect to afferent fibres of the vagus nerve. Lung-irritant receptors are vagal nerve endings situated in the epithelia of the trachea and lower airways. Lung-stretch receptors inhibit inspiratory neurons of the DRG while inspiration is in progress, thereby shortening inspiratory time and reducing the inspiratory tidal volume. Lung-irritant receptors give excitatory input to the DRG, thereby shortening the expiration phase. Together, these vagal afferents produce the typical relatively shallow and rapid breathing pattern that is normal for humans. Removal of these afferents results in a slow and deep breathing pattern (Petersen 2007). Other mechanoreceptors influencing the pattern of breathing are the proprioceptors (joint receptors, Golgi tendon organs and muscle spindles) in the chest wall and diaphragm. These provide information about thoracic inflation and are integrated within the spinal cord, but also relay to the sensory cortex and the respiratory centres (Petersen 2007).

The respiratory system exhibits a number of protective reflexes. One of these is cough, which shall be described in detail in the following section. Other protective reflexes are: sneeze; aspiration reflex; Hering-Breuer reflex; sigh; and the responses to stimulation of bronchial and pulmonary C-receptors. A sneeze is characterized by a number of superimposed inspirations, followed by a strong rapid expiration and then a short pause in the expiratory position, in combination with broncholaryngeal constriction. It is triggered by stimulation of trigeminal nerve endings in the nasal mucosa through chemical or mechanical irritants. The aspiration reflex is a powerful inspiration combined with bronchodilation. It is triggered by mechanical irritation of glossopharyngeal nerve ending in the epithelium of the epipharynx, for example due to a blockage. This response attempts to pull the blockage into the pharynx and thereby re-establish a patent airway. The Hering-Breuer reflex is a response to the degree of lung inflation and is mediated by the lung-stretch receptors. At high levels of inflation, the response is a cessation of

diaphragmatic activity, while at sustained deflation the response is a promotion of strong and frequent inspiratory efforts. A sigh is an augmented breath, which is thought to serve the re-inflation of alveoli that have collapsed during periods of quiet breathing. Sigh is mediated by lung-irritant receptors. Bronchial C-receptors and pulmonary C-receptors (the latter also termed J-receptors) are vagal nerve endings of unmyelinated fibres, situated in the bronchial or alveolar interstitium, respectively. These receptors do not have a role in normal breathing. They are stimulated by mechanical distortion or increases in interstitial fluid pressure, for example through large inflation or lung oedema. The response is broncholaryngeal constriction and apnoea followed by rapid shallow breathing. If the stimulus is severe, the response is the J-reflex, which is an inhibition of the spinal motor neurons. This causes a relaxation of skeletal muscles in response to severe lung damage (Petersen 2007).

1.3.2 Normal cough function

Cough is the most pronounced defensive respiratory manoeuvre. Its function is to generate high expiratory airflow, in order to achieve removal of mucus or other material from the lower airways into the oropharynx. Cough is a reflex function, but can also be initiated voluntarily. Four phases of cough have been described. Cough begins with an inspiratory phase, characterised by an emphasised preparatory inspiration with laryngeal abduction. Then follows the compressive phase, during which the glottis is occluded through laryngeal adductor activity as intra-abdominal and intra-thoracic pressure are built up through expiratory muscle contraction. The third phase of cough, termed expulsive phase, results from a rapid opening of the glottis, achieved by active laryngeal abduction, while expiratory muscles continue to contract. This forceful contraction of expiratory muscles is accompanied by broncholaryngeal constriction, overall resulting in expiratory air flow at high flow rates. It is during this phase that the expectorating effect of cough is exerted. A fourth and final phase is sometimes described as the cessation phase, characterised by a momentary suspension of breathing activity and laryngeal abduction (Widdicombe *et al*. 2011, Petersen 2007, Fontana & Lavorini 2006).

A separate defensive respiratory manoeuvre similar to cough has been described as the (laryngeal) expiration reflex. This is a response to stimulation at the level of the vocal folds or upper trachea. It lacks a distinct inspiratory phase and consists of an immediate glottis compression phase followed by an expulsive phase (Widdicombe *et al*. 2011, Fontana & Lavorini 2006, Widdicombe & Fontana 2006).

Current knowledge about the neural pathways for reflex and voluntary cough in humans is limited. Most evidence is derived from animal models (Brooks 2011, Mazzone 2005). The main reflexogenic zones for cough are the larynx and the tracheobronchial tree. A substantial body of literature describes reflex cough afferents, and various approaches to grouping these have been applied (Canning 2006, Canning *et al*. 2006, Fontana & Lavorini 2006, Singh 2006, Widdicombe 2001, Widdicombe 1998). There is some indication that cough afferents can be distinguished according to function, into mechanosensory and chemosensory. The former consist of myelinated Aβ and Aδ fibres and respond to mechanical stimulation, *i.e.* inhaled foreign materials, and gastric acid; the latter are conveyed partly through Aδ and partly through unmyelinated C-fibres and respond to chemical irritants, such as capsaicin, citric acid and hypertonic saline, and inflammatory processes (Brooks 2011, Mazzone 2005). Sensory afferents from laryngeal receptors are conveyed via the superior laryngeal nerve and terminate in the nucleus tractus solitarii, from where they project further to the pontine and medullary respiratory areas. Similarly, vagal afferents from the lower airways and afferent C-fibres terminate at the nucleus of the tractus solitarius, in a part termed the commissural subnucleus. From there, second-order neurons project extensively to the pontine and medullary respiratory centres. The efferent pathway of reflex cough leads from the medullary respiratory centres (DRG and VRG) via upper motor neurons to the spinal motor neurons supplying the inspiratory and expiratory muscles, effecting the inspiratory and expiratory muscle action; via cranial motor neurons to the intrinsic laryngeal muscles, effecting the co-ordinated laryngeal abduction and adduction; and via sympathetic and parasympathetic efferents to the smooth airway muscles, effecting the bronchoconstriction (Brooks 2011, Bianchi & Gestreau 2009, Fontana & Lavorini 2006). While further details of the neuro-physiology of reflex cough remain to be described, it is suggested that a functional distinction can be made into cough which is triggered mainly through extrapulmonary mechanosensory stimulation and serves as the primary airway

defensive mechanism from aspiration; and cough that is elicited in the context of airway inflammation and disease and is stimulated through intrapulmonary chemosensory afferents (Canning *et al*. 2006, Mazzone 2005).

Current understanding of the neural control of voluntary cough in humans, including the voluntary suppression of reflex cough, is limited. The cortical manifestation of cough sensation is described as “urge to cough” (Brooks 2011). It is thought that areas identified through functional magnetic resonance imaging during volitional inspiration are likely involved in the motor patterns of voluntary cough. These areas include the superior motor cortex, the premotor cortex, the supplementary motor area, the inferolateral sensorimotor cortex, the prefrontal cortex, and the striatum (Fontana & Lavorini 2006). Also, functional magnetic resonance imaging has shown that areas involved in respiratory-related oro-facial tasks (*i.e.* speaking or singing) and areas involved in non-respiratory related oral-facial and tongue movements appear to be involved during volitional coughing (Brooks 2011).

1.3.3 Objective assessment of cough

Cough function can be evaluated in various ways. In clinical situations, cough is mostly evaluated through clinicians’ subjective observation. The presence, frequency and strength of cough may be observed. The quality of the cough sound may be described, and the effectiveness of cough in clearing retained lung secretions may be judged (Jenkins & Tucker 2002).

Objective assessments of cough are mainly conducted in research contexts, whereby various aspects of cough may be evaluated. Methods of measuring reflex cough sensitivity, cough frequency, and cough-related quality of life have attracted increasing interest over the past decade, as the suppression of chronic cough has become a focus for pharmaceutical research (Chung 2006, Irwin *et al*. 2006). In this context, coughing is excessive and disproportionate to the functional benefit of coughing. Cough becomes an irritant and impacts on personal

wellbeing, social interactions and quality of life. Pharmaceutical research of cough-suppressing agents makes use of measures of reflex cough sensitivity, cough frequency, and cough-related quality of life as outcome parameters. These methods are relatively well developed and standardised (Morice *et al*. 2007, Chung 2006). Reflex cough sensitivity is assessed by inhalation cough challenge. The subject is exposed to inhaled chemical irritants, usually citric acid, tartaric acid or capsaicin. The irritant is delivered in increasing concentrations, and the threshold at which reflex coughs are elicited is noted. Cough frequency is measured by counting the number of coughs over a certain period of time. Automated cough monitoring systems have been developed, which greatly reduce the time and effort required to capture cough frequency, particularly over long periods of time. Most systems are based on sound recordings and software packages for the automated detection of cough sounds on such recordings. These systems allow convenient measurement of cough frequency over 24 or 48 hours (Morice *et al*. 2007, Chung 2006). Cough-related quality of life may be assessed using self-reported questionnaires. Several cough-specific measures are available, although generic health-related quality of life questionnaires have also been used (Morice *et al*. 2007, Chung 2006).

Methods of objectively assessing cough intensity or cough strength are of interest in contexts of effective airway clearance or effective protection from aspiration, especially in clinical populations with acquired or degenerative neuromuscular conditions. Here, the focus is on quantifying ineffective cough, for example with the aim of predicting the risk of pneumonia, or to identify the need for assisted airway clearance treatment (Jones *et al*. 2012, Boitano 2006). The loudness of cough sound recordings (sound pressure level) has been used as an indicator for cough strength by some researchers (Smith Hammond *et al*. 2001, 2009). Oesophageal and gastric pressures during a cough manoeuvre can be measured as parameters of cough strength with close relation to respiratory muscle strength, in particular abdominal muscle strength (American Thoracic Society (ATS) & European Respiratory Society (ERS) 2002). Expiratory cough flow is a parameter which most closely relates to the effectiveness of a cough manoeuvre in transporting particles from the lower airways towards and into the pharynx (American Thoracic Society (ATS) & European Respiratory Society (ERS) 2002). It is thought that the sheer forces exerted onto the airway walls through expiratory airflow move mucus and other materials along the airways. In a mechanical model, the distance of material moved was

proportional to the airflow generated (King *et al*. 1985). Cough flow is usually measured at the mouth, using calibrated pneumotachograph systems. Peak expiratory flow during a cough manoeuvre is most frequently taken as the outcome parameter of interest, although various other parameters may be derived form a cough flow recording, such as the peak rise time and the inspired and expired cough volumes (Singh *et al*. 1994). In the context of cough as a defence mechanism from aspiration, the measurement of reflex cough sensitivity is also of interest in neurological conditions such as stroke or traumatic brain injury, where the cough reflex may be diminished due to central depression (Lee *et al*. 2013, Addington *et al*. 2005).

1.3.4 Impairment of cough in acute stroke

Cough function has been an ongoing research interest in neuromuscular conditions. Aspects of cough have been examined in populations with muscular dystrophy (LoMauro *et al*. 2014, Brito *et al*. 2009, Daftary *et al*. 2007, Dohna-Schwake *et al*. 2006, Kang *et al*. 2006a, Gauld & Boynton 2005, Sancho *et al*. 2004), motor neuron disease (Cleary *et al*. 2013, Sancho *et al*. 2007, Bach *et al*. 2006, Bach 1995), spinal cord injury (Yoon *et al*. 2011, Bolser *et al*. 2009, Kang *et al*. 2006b), traumatic brain injury (Lee *et al*. 2013), Parkinson's disease (Silverman *et al*. 2014, Pitts *et al*. 2009) and stroke (Zhou *et al*. 2012, Yoon *et al*. 2011, Ward *et al*. 2010, Smith Hammond *et al*. 2009, Harraf *et al*. 2008, Addington *et al*. 2005, Stephens *et al*. 2003, Smith Hammond *et al*. 2001, Addington *et al*. 1999a, 1999b). Studies of cough in stroke can be distinguished into those that focus on reflex cough sensitivity, and those investigating cough intensity in the sense of cough strength and the generation of cough airflow.

With respect to reflex cough in stroke, there is some evidence indicating that reflex cough sensitivity can be reduced following stroke, and that a diminished cough reflex is associated with an increased risk of pneumonia. Addington *et al*. (1999a) assessed the cough reflex in 161 stroke patients admitted to a rehabilitation hospital within 30 days of stroke. Patients inhaled nebulised L-tartaric acid at a concentration that normally triggers a strong reflex cough response. Thirty patients showed an abnormal (weak or absent) cough reflex at this level of stimulation. Out of these 30 patients, five (17%) developed pneumonia, while none of the

patients with normal cough reflex developed pneumonia. In another publication, the same group reported that cough reflex assessment showed abnormal responses in 40 (10%) out of 400 stroke rehabilitation patients when tested with the nebulised irritant; and 81 (20%) out of 400 patients showed weak or absent voluntary cough as assessed subjectively by the treating clinician (Addington *et al*. 1999b). In their later work, Addington *et al*. observed a cohort of 818 stroke rehabilitation patients whose cough reflex was assessed with the above method. Out of 736 patients with normal cough reflex, 26 (3.5%) developed pneumonia, while out of 82 patients with abnormal cough reflex 9 (11.0%) developed pneumonia (Addington *et al*. 2005). The group also conducted a study of the laterality of voluntary and reflex cough in 30 right-handed patients with acute middle cerebral artery infarction (Stephens *et al*. 2003). Cough reflex, as assessed through inhalation challenge using L-tartaric acid, and voluntary cough, assessed subjectively by a clinician, were both normal in all 16 patients with right hemispheric lesion sites. Out of 14 patients with left hemispheric stroke lesions, reflex cough was normal, and voluntary cough was weak or absent in 11 patients (79%). None of the patients in this study developed pneumonia. The authors hypothesised that the neural control of voluntary cough might be closely linked to speech and voice-clearing, which might account for the observed impairment of voluntary cough in patients with left-sided stroke lesions (Stephens *et al*. 2003). Overall, these studies provide some evidence that reflex cough sensitivity can be considerably reduced in a portion of acute and sub-acute stroke survivors; and that an abnormal cough reflex is associated with increased incidence of PSP.

Several studies have examined cough intensity in stroke using cough flow measurements. Smith Hammond *et al*. conducted two cross-sectional studies, examining the relationship between cough flow parameters and swallowing function in stroke (Smith Hammond *et al*. 2009, 2001). Both studies applied similar methods. Voluntary cough flow was recorded using a face mask and the Perci-SAR system, a customised speech-aeromechanic measurement system that includes a pneumotachograph (MicroTronics Corp, Chapel Hill, North Carolina). Sound pressure levels were recorded using a calibrated microphone. Instrumental swallowing assessments (video-fluoroscopy or fibre-optic endoscopic evaluation of swallow) were conducted to determine whether patients aspirated or not. In their first study, Smith Hammond *et al*. compared 43 acute and sub-acute stroke patients with 18 age- and sex-matched healthy

control subjects. Compared with the control subjects, stroke patients had reduced parameters of cough intensity throughout. These parameters were: duration, volume and peak flow of the inspiratory phase of cough; duration of the glottis compression phase of cough; peak flow and cough volume acceleration during the expulsive phase of cough; and cough sound pressure levels. Within the stroke sample, these parameters generally decreased with worsening degree of swallowing dysfunction and aspiration (Smith Hammond *et al*. 2001). In their second study, Smith Hammond *et al*. assessed 96 stroke patients using similar methods and replicated their findings of generally worse cough function in patients who aspirated compared with non-aspirators. In both studies, the authors also analysed the diagnostic value of voluntary cough flow measurement for the identification of aspiration risk in stroke patients. Their findings support the association between reduced cough flow parameters and aspiration, and they suggest that further research could be conducted to validate the use of objective measures of voluntary cough for the assessment of aspiration in stroke (Smith Hammond *et al*. 2009).

Further evidence of the impairment of cough flow in stroke was provided by Yoon *et al*. (2011). In their study, 31 chronic stroke patients were compared with 30 healthy elderly subjects. Peak expiratory flow rate of voluntary cough was found to be reduced by approximately one third in the stroke group. Similarly, Zhou *et al*. (2012) found that in a group of 32 acute stroke patients voluntary peak cough flow rate was reduced by approximately one third when compared with the normative range for peak expiratory flow (during forced expiration).

The studies by Ward *et al*. (2010) and Harraf *et al*. (2008) provide the most detailed and physiologically sophisticated examinations of cough and respiratory muscle function in stroke to date. Ward *et al*. studied 18 patients with acute middle cerebral artery infarcts in comparison with 20 age-matched healthy control subjects. Subjects did not have any respiratory conditions or neuromuscular conditions affecting the respiratory pump, other than stroke. Investigations included assessments of lung function (forced spirometry), respiratory muscle strength (gastric and oesophageal pressures, inspiratory and expiratory mouth pressures, and sniff pressures), and cough flow for voluntary and reflex cough measured with a pneumotachograph at the mouth. Reflex cough was triggered through nebulised L-tartaric acid solutions of escalating

concentrations. Stroke patients had significantly reduced respiratory muscle strength and cough flow, for both voluntary and reflex cough, with parameters reduced by one third to half in comparison with the control group.

Several aspects of the study by Ward *et al*. are noteworthy. Firstly, the observation that in addition to voluntary cough, reflex cough intensity was also reduced in this group of hemispheric stroke patients may seem unexpected. Intuitively, it could be assumed that reflex cough function, which is primarily controlled at the level of the brainstem, would not be affected in hemispheric stroke; however, these findings suggest otherwise, and on this basis hypotheses of the central neural mechanisms involved in reflex cough may be reconsidered. Secondly, the authors were able to exclude any undetected obstructive airway disease in their subjects, by conducting forced spirometry. Airway obstruction limits forced expiratory airflow, and therefore also cough expiratory airflow. The subjects studied by Ward *et al*. all had parameters of airway obstruction (FEV_1/FVC ratio) within the normal to mildly obstructive range, and there was no significant difference between the stroke patients and control subjects. It can therefore be asserted with confidence that the cough flow limitations in the stroke group observed by Ward *et al*. were not related to airway obstruction, but to other aspects of cough generation. Thirdly, the study gives a good indication as to which physiological impairments result in the observed cough dysfunction. From the point of view of cough mechanics, the volume inspired at the beginning of cough, the driving pressure generated by expiratory muscle contraction, and the quality of glottis control (adequate glottis closure during the compression phase of cough and rapid glottis opening to initiate the expulsive phase) all contribute to effective cough generation. Stroke patients showed adequate cough compression phases as visualised on cough flow-time traces, and no significant difference in glottis closure times compared with control subjects, indicating adequate glottis function during cough. However, stroke patients showed a reduction in inspiratory lung volumes (FVC, inspiratory cough volume) and in both in- and expiratory muscle strength (maximum inspiratory and expiratory mouth pressures, sniff pressures, gastric and oesophageal cough pressures). It can therefore be surmised that the following two factors are the likely causes of reduced cough intensity in acute stroke: 1) reduced cough inspiratory volume, related to reduced inspiratory muscle strength; and 2) reduced cough driving pressures, related to reduced expiratory muscle strength.

The study by Harraf *et al*. (2008) provided evidence to identify the site of neuro-muscular impairment causing reduced cough in stroke. Comparing 15 patients with acute hemispheric infarct and 16 age- and sex-matched controls, Harraf *et al*. studied voluntary cough flow, respiratory muscle strength (maximum expiratory mouth pressure, gastric and oesophageal cough pressures), and in- and expiratory muscle activation by transcutaneous magnetic stimulation (TMS). As in the study by Ward *et al*., expiratory muscle strength and expiratory cough flow were reduced by one third to half in the stroke group compared to healthy control subjects. Harraf *et al*. then measured gastric and oesophageal pressures when respiratory muscles were stimulated involuntarily through magnetic stimulation. The diaphragm was activated through peripheral phrenic nerve stimulation from the anterior neck. The abdominal musculature was activated through stimulation of the spinal nerve root at the tenth thoracic level. These stimulations resulted in equivalent gastric and oesophageal pressures in the stroke and control group. However, when the abdominal musculature was activated through TMS over the vertex, the stroke group showed lower pressures than the control group. This indicates that the function of the peripheral portion of the motor unit (neural transmission along the lower motor neuron and trigger of muscle contraction) in stroke is equivalent to neurologically normal controls; and that the breakdown in respiratory muscle force generation observed in stroke is likely related to the central portion of the motor unit, *i.e.* the cerebral lesion.

In summary, there is good evidence to demonstrate that there is a significant impairment of cough and respiratory muscle function in acute stroke; that the impairment in cough function is likely related to respiratory muscle weakness, as opposed to dysfunction at the level of the glottis; and that the respiratory muscle weakness is related to the central component of the motor pathway, *i.e.* the stroke lesion. This provides the physiological rationale for the novel treatment approach taken in the present studies, which is to target stroke-induced central respiratory muscle weakness to improve cough effectiveness.

1.4 Respiratory muscle training

Approaches to training the respiratory muscles follow the general principles of skeletal muscle training, which is founded on exercise physiology, although adapted to the functional requirements of the respiratory system (McConnell 2013, pp. 135-147, Syabbalo 1998, Polkey *et al*. 1995, Reid & Dechman 1995). These principles are overload, specificity and reversibility (McConnell 2013, pp. 135-147, 2011, pp. 80-85). Overload refers to imposing a training challenge that exceeds the usual level of muscle performance. Implicit within this principle is the concept of training duration, intensity and frequency. Accordingly, an overload can be applied to a muscle by increasing the duration, intensity or frequency the muscle is required to contract at, whereby most training regimens combine these factors (McConnell 2011, p. 80). Specificity refers to the principle that the nature of the training response depends on the type of stimulus delivered. Here, the general distinction between strength and endurance training is made. High intensity and short duration training stimuli lead to an improvement in strength, whereas low intensity and long duration stimuli lead to an improvement in endurance (McConnell 2011, p. 82). The reversibility principle refers to the phenomenon of detraining. Training gains need to be maintained through ongoing use, or else they will be reversed ('use it or lose it'). Generally, a maintenance programme with stimuli at a lower level than for the training programme is sufficient to prevent the loss of training gains (McConnell 2011, p. 84).

Approaches to training the respiratory muscles can be divided according to the desired training response into resistance training (aimed at improving strength) and endurance training. Another distinction can be made into functional training of the respiratory muscles, *i.e.* training the respiratory muscles through breathing-based training activities, and traditional skeletal muscle training, *i.e.* training respiratory muscles through traditional weight-lifting type exercises, which is possible for the abdominal muscles and some of the accessory respiratory muscles. Functional RMT can be divided according to the 'direction' of training, *i.e.* inspiratory or expiratory training. A further distinction into RMT and other respiratory-type exercises and novel interventions can be made, mainly based on what historically has been understood and described as RMT in the literature, and there may be some overlap with respect to the proposed

mechanisms of effect and aims of training. Other respiratory-type exercises and novel interventions include diaphragmatic breathing (Sutbeyaz *et al*. 2010), pursed lip breathing (Sutbeyaz *et al*. 2010), directed breathing with biofeedback (Kim *et al*. 2011) and electrical stimulation of respiratory muscles (Jung *et al*. 2014).

The following sections describe techniques that historically have been described as RMT in the literature. Most robust evidence is derived from research in healthy and athletic populations (McConnell & Romer 2004) and a limited but growing body of evidence is available for several clinical populations (McConnell 2013, pp. 161-162). Historically, most work has focussed on training of the inspiratory muscles, as opposed to the expiratory muscles (McConnell 2013, p. 140).

1.4.1 Voluntary isocapnic hyperventilation training

Voluntary isocapnic hyperpnoea is an endurance training method that involves maintaining voluntary hyperventilation at 60% to 90% of maximum voluntary ventilation for up to 40 minutes. Sustained hyperventilation in healthy subjects leads to hypocapnia with tingling sensations in hands, feet and lips, dizziness and eventual loss of consciousness (Martini 2004, p. 1036). In voluntary isocapnic hyperventilation, hypocapnia is avoided by the use of a rebreathing circuit, which allows the subject to maintain hyperventilation over an extended period of time. This requires an elaborate equipment setup. Voluntary isocapnic ventilation leads to improvement in respiratory muscle endurance but not strength (McConnell 2013, pp. 140-141, 2011, pp. 85-92, Verges *et al*. 2008).

1.4.2 Static resistive load training

In early studies of respiratory muscle strength training, training stimuli were applied through ‘quasi-isometric’ static contractions of the inspiratory or expiratory muscles, whereby subjects

exerted an inspiratory or expiratory effort against a closed lumen (Reid & Dechman 1995, Leith & Bradley 1976). Although this training method was shown to increase respiratory muscle strength (Leith & Bradley 1976), alternative methods that require subjects to shift lung volume were regarded as more functional and have been favoured training methods in recent years (Reid & Dechman 1995).

1.4.3 Flow resistive load training

In flow resistive load training, the training stimulus is achieved by breathing through a device with a narrowed lumen, whereby the size of the lumen can be selected to adjust the training intensity. As airway resistance is dependent on both the diameter of the airway and airflow, resistance increases with both decreasing airway diameter and increasing airflow. In theory, training resistance to the respiratory muscles can conveniently be applied by reducing the lumen of the airway while maintaining airflow constant. The principle underlying this method is simple; however, effective training requires monitoring of the airflow (*i.e.* the speed of breathing), as slower breaths result in reduced resistance and therefore lower training intensity (McConnell 2013, p. 139, 2011, p. 86, Reid & Samrai 1995).

1.4.4 Pressure threshold loading

In the pressure threshold loading method, the training stimulus is delivered through a device containing a spring-loaded valve, which opens at a pre-set pressure. For inspiratory training, the subject breathes in against the threshold load and has to generate sufficient inspiratory pressure to open the valve, from which point on the inspiration can be completed. The breath out is unimpeded. For expiratory training, the direction of the spring-loaded valve is reversed (McConnell 2013, pp.139-140, 2011, pp. 86-87, Reid & Samrai 1995). The load can be adjusted conveniently by tightening or releasing the spring. This training method has become increasingly popular, due to the relatively simple and inexpensive training devices that are nowadays available. A further advantage of this method is that the training load is not influenced

by airflow (*i.e.* the same threshold load is delivered, regardless whether the subjects breathes through the valve at higher or lower airflow); and that the function of the device is not dependent on gravity (*i.e.* the device can be tilted in any direction, making it convenient for use with patients who are in bed, as opposed to other respiratory training devices which require upright positioning for the device to function adequately) (McConnell 2011, pp. 86-87, Reid & Samrai 1995).

1.4.5 Physiological responses to pressure threshold loading

The physiological responses to RMT using pressure threshold loading have been researched extensively in healthy and athletic populations. Inspiratory muscle training has been shown to lead to inspiratory muscle hypertrophy and improvement in strength (McConnell 2013, pp. 97-131, 2011, p.52-56, McConnell & Romer 2004). In general, respiratory muscles respond to high-load low-frequency loading by increase in strength. Taking into account both the resistance load and the airflow generated during a training manoeuvre, it has been shown that training with high loads at low airflow leads to improvement in maximal strength but not maximal flow. Training with low loads at high flow leads to improvement in maximal flow but not strength. However, training stimuli at intermediate loads and flow elicit improvements in both maximal strength and flow (Romer & McConnell 2003). Accordingly, the recommended training load in healthy subjects is at 50% to 70% of maximal respiratory muscle strength, and with a frequency of 30 breaths at least twice daily (McDonnell 2011, pp. 93-111). In addition to the training effects on the respiratory system, transient increase in heart rate and arterial blood pressure has been observed in healthy athletes in response to inspiratory threshold training at a moderate resistance load (60% of maximal inspiratory mouth pressure; PImax) (McConnell & Griffiths 2010).

In clinical populations, RMT has been researched most in patients with primary cardio-respiratory conditions and in surgical patients, and some studies have been conducted in patients with neuromuscular conditions (McConnell 2013, pp. 135-173, Pollock *et al*. 2013, Hulzebos *et al*. 2012, Lin *et al*. 2012, Gosselink *et al*. 2011). Most consistently, studies have

demonstrated that inspiratory muscle training leads to improvements in maximal inspiratory mouth pressure, inspiratory muscle endurance and subjective perception of dyspnoea. Some studies have also demonstrated improvements in lung function (spirometry) and exercise tolerance (McConnell 2013, pp. 135-173).

1.5 Systematic literature review of respiratory muscle training in stroke

A systematic literature review was conducted to evaluate the evidence base for RMT in stroke; and to use previous research in the field to inform the subsequent clinical studies.

1.5.1 Search strategy

Fourteen databases were searched between September and November 2012, using the search terms ‘stroke’ in combination with ‘respiratory training’ and ‘respiratory exercise’. Details of the search strategy and search history are given in Table 2 and Figure 1. Included were research articles or conference abstracts of studies investigating RMT in stroke patients, which were published in indexed, peer-reviewed scientific journals. To maximise the pool of potential results, no limitations were set with respect to time of publication, language or study design. The search yielded 2,928 results. Where the title of the search result appeared relevant, the abstract was read. Where the abstract appeared relevant, the citation was obtained in full text. Reference lists of citations obtained in full text were reviewed for further relevant publications. Eight citations were obtained in full text, of which three were excluded: one conference abstract (Teixeira-Salmela *et al*. 2010), which describes the study published in full by Britto *et al*. (2011); and two reports of clinical studies, which investigated respiratory exercise other than RMT (Kang *et al*. 2012; Kim *et al*. 2011). Of the remaining five publications, three are reports of original research (Britto *et al*. 2011, Sutbeyaz *et al*. 2010, Fernandes *et al*. 2007), and two are systematic reviews of RMT in stroke (Pollock *et al*. 2012; Xiao *et al*. 2012), each of which

included two of the three original research articles identified (Britto *et al*. 2011, Sutbeyaz *et al*. 2010). Monthly automated search alerts were set up to identify newly published articles, and one further study was added for review in May 2014 (Jung *et al*. 2014). The literature search is up to date as of 31st December 2014.

Due to the paucity of available publications, the value of statistically combining the reported results is limited. Rather, detailed appraisals of individual studies are warranted, which are given in the following sections.

Table 2. Search strategy for the systematic literature search

Database (search portal)	Date	Search terms	Database fields	Results
MEDLINE (PubMed)	17/09/2012	stroke AND respiratory training	Title, abstract	1
	17/09/2012	stroke AND respiratory training	All fields	181
BIOSIS, SciELO, Web of Science Core Collection (Web of Knowledge v. 5.7)	18/09/2012	stroke AND *piratory AND (train* OR exercise*) NOT swim* NOT cardio*	Topic	373
CINAHL (EBSCO Host)	12/11/2012	stroke AND resp* AND (train* OR exercise*)	All fields	962
Embase, PsychINFO (OvidSP)	12/11/2012	stroke AND respir* AND (train* OR exercise*)	All fields	804
AMED, BNI (NHS Evidence)	13/11/2012	stroke AND respir* AND (train* OR exercise*)	Title, abstract	20
	13/11/2012	stroke AND respiratory	Title, abstract	51
PEDro, OTSeeker, PsycBITE, SpeechBITE (Allied Health Evidence)	13/11/2012	stroke respiratory	All fields	29
	13/11/2012	stroke breathing	All fields	10
	13/11/2012	stroke AND resp* AND (train* OR exercise*)	All fields	0
	13/11/2012	stroke respiratory train*	All fields	6
metaRegister of Controlled Trials (www.controlled-trials.com)	13/11/2012	stroke AND respiratory	All fields	491

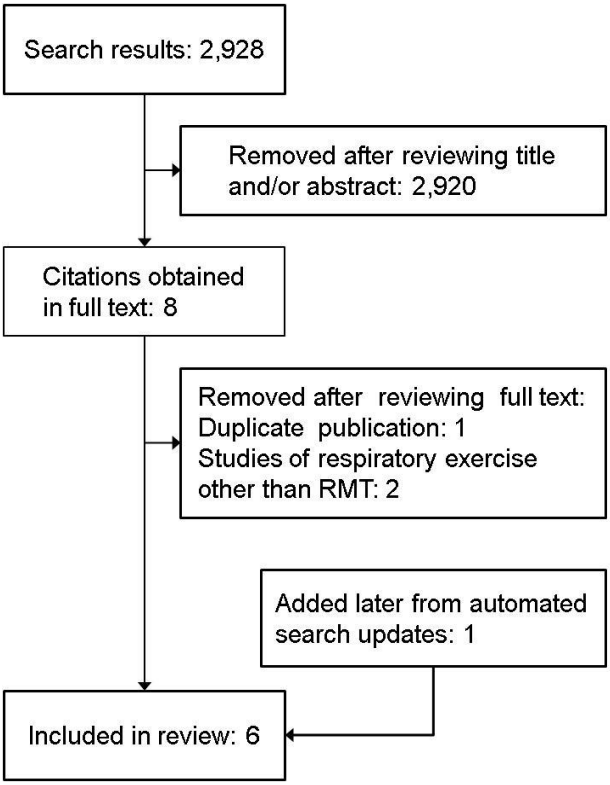


Figure 1. Flow diagram of the systematic literature search

1.5.2 Critical appraisal of individual studies

1.5.2.1 Inspiratory Muscular Training in Chronic Stroke Survivors: A Randomized Controlled Trial (Britto *et al*. 2011)

Britto *et al*. (2011) conducted a randomised controlled trial of RMT in 21 chronic stroke survivors. The study recruited community-dwelling adults in a large Brazilian city. Criteria for recruitment were: a history of stroke more than nine months prior to enrolment; presence of a residual hemiparesis; maximal inspiratory mouth pressure (PImax) of less than 90% of that predicted for age and gender; and lack of pre-existing respiratory or cardiac conditions. Participants were randomised to receive either pressure threshold training RMT with a

commercially available inspiratory muscle training (IMT) device (Threshold IMT, Philips Respironics, Andover, MA) or sham respiratory training.

RMT was conducted over a period of eight weeks. Participants trained independently and completed a training diary. The training resistance for the intervention group was set at 30% of PImax, and adjusted in biweekly sessions with a researcher. The control group followed the same procedures as the intervention group, but used training devices without valves, thereby breathing through the device without added resistance. Participants and assessors were blinded. The primary outcome was PImax. Further outcomes were inspiratory muscle endurance, cardiovascular fitness, daily functioning and quality of life. The data was examined for normality and repeated-measures analysis of variance (ANOVA) was used for the inferential statistical analysis.

After eight weeks of training, both groups showed an increase in mean PImax (Table 3). This improvement was three times higher in the intervention group, with a statistically significant between-group difference (p=0.033). The intervention group also demonstrated a statistically significant increase in inspiratory muscle endurance (p=0.04), while there was a minimal and statistically insignificant increase only in the control group. In the remaining three outcomes, there was no treatment effect in both groups.

Table 3. Values for outcome maximal inspiratory mouth pressure (PImax, cmH_2O) in the study by Britto *et al.* (2011)

Group	n	Baseline	Post intervention	Change score	95% CI for change score [a]
IMT	9	67.8 (14.6)	102.2 (26.0)	34.4 (27.1)	13.6, 55.2
Control	9	45.6 (13.8)	56.7 (8.7)	11.1 (2.9)	8.9, 13. 3

Figures are mean (SD)
IMT, inspiratory muscle training.
[a] 95% confidence intervals for change scores are not given in the paper and were calculated from the given mean (SD)

As a double-blind randomised controlled trial, the study of Britto *et al*. had a low risk of bias. The inclusion of a sham intervention and transparent statistical methods further strengthened the design, and a relatively high level of confidence can be placed in the reported results. The study benefited from an explicit hypothesis statement and a clear primary outcome of interest, PImax. A sample size calculation was conducted to detect an effect size of 12.3 cmH_2O in PImax. In spite of three participants who did not complete the study, the sample of nine participants per group met the requirements of the sample size calculation to give 80% power at a significance level of 5%.

One negative criticism relates to the group characteristics at baseline. Clinical characteristics relating to stroke were not reported, such as the type of stroke, the time from the onset of stroke, or the severity of hemiparesis. These data would add to the reader's understanding of the sample and the potential transferability of findings. Although the authors were satisfied that the two groups were sufficiently similar at baseline, several group differences are evident from the reported baseline characteristics: body mass index, PImax, inspiratory muscle endurance, and cardiovascular fitness were considerably higher in the intervention group. While it is uncertain whether these differences would confound the study findings, these differences are obvious and should have been acknowledged by the authors.

The authors present summary data for all five outcomes at baseline and post-training. Also presented are within-group differences (change scores) and between-group differences (differences in change scores) for all outcomes. The 95% confidence interval for the between-group difference in PImax change score was 0.9 to 45.8 cmH_2O. Accordingly, the true difference in treatment effect of IMT on PImax compared to sham IMT could be as large as 45.8 cmH_2O or as little as 0.9 cmH_2O. While at the upper end of the confidence interval this represents a highly relevant effect size, at the lower end of the confidence interval the value approaches the effect size observed in the control group. It is advisable to bear this wide confidence interval in mind when judging the clinical relevance of the findings. No long-term follow-up was carried out, and therefore no data is available on whether the training effect was maintained after RMT was discontinued.

In summary, the study by Britto *et al*. warrants confidence in the reported findings. Although there were some group differences at baseline, it is uncertain whether these could have confounded the results, and it is reasonable to attribute the change in outcome measures to the intervention. The study recruited a sufficiently large sample to reach the statistical power aimed for; however, the confidence intervals remain wide and at the lower margin the difference between intervention and control group is minimal. Accordingly, it can be concluded that IMT in chronic stroke survivors appears a promising intervention to increase inspiratory muscle strength and endurance. However, these findings are preliminary and warrant further research to improve the statistical confidence in the findings, which will be achieved through larger samples.

1.5.2.2 Respiratory muscle training improves cardiopulmonary function and exercise tolerance in subjects with subacute stroke: a randomized controlled trial (Sutbeyaz *et al*. 2010)

Sutbeyaz *et al*. (2010) conducted a randomised controlled trial in 45 subacute stroke patients. Adults with a first ever hemiparetic stroke were recruited from a Turkish inpatient rehabilitation unit on average five months after onset of stroke. Patients with pre-existing respiratory or cardiovascular conditions were excluded. Participants were randomised into three groups. The first experimental group carried out 'breathing retraining exercises' (BRT), a combination of diaphragmatic breathing, pursed lip breathing, air-shifting techniques, and voluntary isocapnic hyperpnoea; the second experimental group carried out IMT using pressure threshold loading with a commercially available device (Threshold IMT, Philips Respironics, Andover, MA); the control group received no specific respiratory exercise.

Interventions were delivered over a period of six weeks, during which all participants underwent the usual post-stroke rehabilitation programme. A number of outcome measures were assessed: lung function testing, cardiovascular fitness testing, activities of daily living, perceived dyspnoea on exertion, and health-related quality of life. Overall, the authors analysed 18 different outcome parameters. Following testing for normality of the data, the authors used one-

way ANOVA for inferential statistical analysis. The most sizeable treatment effect was observed in the IMT group, which showed statistically significant improvements in lung function, including a mean increase in PImax of 7.9 cmH_2O ($p<0.001$), and cardiovascular fitness, including a mean increase in power output of 9.0 W during an incremental arm crank ergometer test ($p=0.01$). The BRT group also showed some treatment effect, with a statistically significant mean increase in PImax of 7.1 cmH_2O ($p<0.001$), in maximal expiratory mouth pressure (PEmax) of 5.4 cmH_2O ($p<0.001$), and in peak expiratory flow (PEF) of 0.6 L/s ($p=0.01$). There was insignificant or no improvement in other parameters in the BRT group. The mean increase in ergometer power output in the BRT group was 1.8 W. The control group showed statistically insignificant improvements in PImax, maximal expiratory mouth pressure (PEmax) and ergometer power output, and no change in the remaining parameters. Table 4 summarises maximal mouth pressure values for the study sample.

Table 4. Values for outcomes maximal inspiratory and expiratory mouth pressure (PImax, PEmax) in the study by Sutbeyaz *et al.* (2010)

Variables	Group	n	Baseline	Post intervention	Change score	95% CI for change score [a]
PImax (cmH_2O)	BRT	15	50.3 (6.7)	57.3 (7.9)	7.1 (4.8)	4.4, 9.7
	IMT	15	49.5 (5.9)	57.3 (8.6)	7.9 (6.6)	4.2, 11.5
	Control	15	51.0 (6.3)	53.9 (6.3)	2.9 (1.9)	1.8, 4.0
PEmax (cmH_2O)	BRT	15	60.8 (7.1)	66.2 (8.2)	5.4 (2.9)	3.8, 7.0
	IMT	15	60.7 (9.2)	62.8 (9.9)	2.1 (2.0)	1.0, 3.2
	Control	15	62.9 (6.4)	65.9 (5.7)	3.0 (1.6)	2.1, 3.9

Figures are mean (SD)
BRT, breathing retraining; IMT, inspiratory muscle training.
[a] 95% confidence intervals for change scores are not given in the paper and were calculated from the given mean (SD)

The study by Sutbeyaz *et al*. holds several strengths, but also has a number of limitations. As a randomised controlled trial, it provides high level evidence regarding the effect of the investigated interventions, although only the assessors were blinded. Blinding participants, for example using a sham intervention for the control group, would have further strengthened the

design. The randomisation procedure was carried out appropriately, and the three groups showed comparable baseline characteristics.

The authors state that a sample size calculation was conducted to detect a 20% difference in improvement between groups with a power of 80% and a significance level of 5%. However, the outcome parameter used in the power calculation is not made explicit. The study would have benefited from a clearly stated hypothesis defining the primary outcome of interest. Accordingly, the statistical analysis method appears unfocused, with between-group comparisons of change scores and within-group comparisons of pre- and post-intervention values conducted for each of the 18 outcome parameters. Although it is reported that adjustment for multiple testing was carried out, this is not made evident in the presented results.

Confidence intervals are not presented, but can be calculated from the data provided in order to estimate the precision of the results. For example, the 95% confidence interval for the mean increase in PImax in the IMT group was 4.5 to 11.3 cmH_2O. The mean difference in PImax change score between the IMT group and the control group was 5.0 cmH_2O, with the 95% confidence interval ranging from 4.5 to 5.4 cmH_2O. This is a narrow confidence interval indicating that in subacute stroke survivors undergoing IMT, with 95% probability the mean increase in PImax is between 4.5 and 5.4 cmH_2O higher than in stroke patients who do not carry out any respiratory exercise. The study did not include long-term follow up and therefore no data is available on whether the positive treatment effects were maintained after the intervention was discontinued.

Two aspects stand out amongst the results. Firstly, both experimental treatments resulted in a similar increase in PImax compared to the control group. In addition, the BRT group also showed an increase in PEmax and PEF, while the IMT group had PEmax and PEF changes similar to the control group. It therefore seems that the combination of breathing exercises delivered to the BRT group had an effect on lung function parameters that was similar, if not more beneficial, to the IMT group. The lack of improvement in PEmax and PEF in the IMT group

may be explained through the mechanics of the training device, which delivers resistance during the inspiration phase only, while components of BRT created increased expiratory resistance (pursed lip breathing and isocapnic hyperpnoea), which could act as a training stimulus for the expiratory muscles and impact on expiratory function. Secondly, only the IMT group showed improvements in those parameters that relate to respiratory muscle endurance (maximum voluntary ventilation, MVV), and cardiovascular fitness (peak oxygen consumption, VO_2peak; power output during incremental arm crank ergometer test; perceived dyspnoea on exertion). This may indicate that, while both interventions are effective in increasing PImax, an indicator of inspiratory muscle strength, IMT also has an effect on respiratory muscle endurance and overall cardiovascular fitness. This finding is consistent with trials of IMT in other clinical populations.

In summary, the study by Sutbeyaz *et al*. merits confidence in the presented results due to a strong study design. However, lack of clarity in the inferential data analysis method and overall modest positive effect sizes warrant caution with respect to the statistical significance and clinical relevance of these findings.

1.5.2.3 Efeito do Treinamento Muscular Respiratorio por Meio do Manovacuometro e do Threshold Pep em Pacientes Hemipareticos Hospitalizados [Effect of Respiratory Muscle Training through Vacuum-Manometer and Threshold PEP in Hospitalised Hemiparetic Patients] (Fernandes *et al*. 2007)

Fernandes *et al*. (2007) conducted a clinical study of 36 adult inpatients with a diagnosis of stroke and hemiparesis. Patients were recruited from a hospital in a large Brazilian city. Participants were divided into two groups of 18. The intervention group received daily expiratory muscle training using pressure threshold loading with a commercially available device (Threshold PEP, Philips Respironics, Andover, MA) at a resistance level of 40% of PEmax over five days. The control group did not receive any respiratory training. Both groups underwent respiratory testing of PImax, PEmax and PEF at baseline and after five days.

The authors present summary data for each of the three outcomes at baseline and at day five, but no change scores or differences in change scores (Table 5). The data were analysed using ANOVA and with the level of significance set at p=0.05. Overall, the intervention group showed statistically significant improvement in PEmax (p<0.001), PImax (p=0.003) and PEF (p=0.021). The control group showed deterioration in all three outcomes.

Table 5. Values for outcomes maximal inspiratory and expiratory mouth pressure (PImax, PEmax) in the study by Fernandes *et al.* (2007)

Outcome	Group	n	Baseline	Post intervention	Mean change score [a]
PImax	EMT	18	39 (14)	56 (20)	17
(cmH_2O)	Control	18	45 (14)	37 (11)	-8
PEmax	EMT	18	43 (16)	70 (19)	27
(cmH_2O)	Control	18	56 (17)	46 (15)	-10

Figures are mean (SD)
EMT, expiratory muscle training
[a] Mean change scores are not given by the authors and were calculated as mean score post intervention minus mean score at baseline; the standard deviations of mean change scores cannot be derived from the data reported in the paper, and therefore 95% confidence intervals for change scores cannot be calculated.

In the publication by Fernandes *et al*. some aspects of the study methodology were described in sufficient detail to support the validity of the findings. For example, the respiratory testing methods were described in detail and comply with the international standards set out by the American and European respiratory societies. However, the authors did not state key aspects of their methodology, most importantly how participants were assigned to the intervention and control group and the degree of blinding of assessors, participants and health care staff. Also lacking are descriptions of a power calculation; detailed inclusion and exclusion criteria; participants' baseline characteristics, including demographic and stroke-related clinical data; the participant flow through the study and participant attrition; the study setting; the level of additional contact with health care or research staff for the two groups as a result of involvement in the study; and whether an adjustment for multiple testing was incorporated in the statistical analysis. Providing change scores and differences in change scores with 95% confidence

intervals would have aided the interpretation of results, in particular with respect to estimating the potential effect size and its clinical relevance. From the data given, confidence intervals cannot be calculated. The authors were contacted with a request for further information, however no reply was received.

In summary, due to the lack of detail in the research paper by Fernandes *et al*., it is not possible to determine the risk of bias in the study. Subsequently, no judgement can be made on the internal validity of the reported findings. While, in view of the paucity of research on RMT in stroke, it is relevant to include the study by Fernandes *et al*. in this review, these findings should not be used towards the cumulated evidence.

1.5.2.4 Effects of abdominal stimulation during inspiratory muscle training on respiratory function of chronic stroke patients (Jung *et al*. 2014)

This South Korean study compared IMT using pressure threshold loading with IMT augmented with concurrent electrical stimulation of the abdominal muscles. The aim was to determine the effect of abdominal stimulation in addition to IMT on forced expiratory function. Eighteen chronic stroke survivors with hemiplegia were recruited and the average time from stroke onset was four years. Participants were randomised to the two training conditions, which were delivered for 20 minutes per day, three times per week over four weeks. IMT resistance was set at 30% of PImax. Outcome measures were diaphragm thickness ratio and forced spirometry, which were assessed before and after the intervention.

The authors observed improved diaphragm thickness ratios at the post-training time point in both groups, with no between-group difference. With respect to forced expiratory function (forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), PEF, and forced expiratory flow between 25% and 75% of the expired volume (FEF_{25-75})), the IMT plus abdominal stimulation group showed improvement throughout, whereas the IMT group remained stable, with statistically significant between-group differences for FEV_1 and PEF. The

authors conclude that, while IMT benefits inspiratory lung function, abdominal stimulation in addition to IMT has the potential to improve lung function parameters relating to forced expiration, such as forced expiratory flow.

The study by Jung *et al*. is interesting in its original and novel approach; however, it is of limited relevance to the present research. Firstly, the experimental component of the intervention was electrical stimulation of the abdominal muscles to augment RMT, as opposed to RMT alone. Electrical stimulation techniques of respiratory muscles have mainly been researched in clinical populations with spinal cord injuries (Golle *et al*. 2008), with the aim to improve tidal volume, forced expiratory function and cough function. While electrical stimulation may merit further investigation in the stroke population, also in the context of improving cough function, the focus of the present studies is RMT alone. Secondly, several methodological aspects of the study by Jung *et al*. could not be assessed due to lack of reporting. Participant baseline characteristics were not reported according to study group, and there was no indication of the severity of stroke impairment or the presence of pulmonary disease. The authors did not state the concealment of group allocation or the level of blinding. While from the data presented it can be assumed that all participants remained in the study until the post-test time point, treatment fidelity was not described. Of note, the outcome parameters relating to forced spirometry (FVC, FEV_1, PEF, FEF_{25-75}) were reported as percentage of the predicted value, rather than in the original unit of measurement. This is unusual and raises the question whether this statistic may have been selected in order to present the findings more favourably.

In summary, the study by Jung *et al*. investigated a novel treatment approach in combining electrical stimulation with RMT. However, this intervention has little relevance in the context of the present studies. Also, several methodological aspects of the study have not been reported, and a judgement on risk of bias and internal validity is not possible. The study should therefore not be included towards the cumulative evidence of RMT in stroke.

1.5.2.5 Respiratory muscle strength and training in stroke and neurology: a systematic review (Pollock *et al*. 2012) and Inspiratory muscle training for the recovery of function after stroke (Xiao *et al*. 2012)

Two systematic reviews of RMT in stroke have been published. The review by Xiao *et al*. (2012), published in the Cochrane Database of Systematic Reviews, included randomised controlled trials of inspiratory RMT in stroke. The review by Pollock *et al*. (2012) included randomised controlled trials of in- and/or expiratory RMT in stroke. Due to the paucity of published studies, Pollock *et al*. extended their review to include populations with various neurological conditions, including Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, and myasthenia gravis, and conducted a meta-analysis of RMT in neurological populations. This approach is possible on the basis that common outcome measures (maximal mouth pressures) were applied in all included studies; although the clinical populations differ in that stroke represents a one-off insult to the brain with remarkable potential for recovery, and the other included conditions are generally of the progressive degenerative-type.

In both reviews only two randomised controlled trials of RMT in stroke could be identified (Britto *et al*. 2011, Sutbeyaz *et al*. 2010). No studies of RMT in stroke using different research designs were identified. The combined effect size of IMT on PImax in the meta-analysis by Pollock *et al*. was a mean (95% CI) group difference of 6.9 (1.8, 12.1) cmH_2O for the two studies in stroke patients, and 6.9 (2.8, 11.0) cmH_2O for all nine studies including different neurological conditions. In relation to absolute values of PImax in healthy and clinical populations, this can be considered a modest effect size.

A note on different statistical analysis methods between the original research studies and the systematic reviews of these studies shall be made at this point. The authors of the original studies analysed the mean differences (change scores) in outcome parameters from pre- to post-intervention. In contrast, the reviews by Xiao *et al*. and Pollock *et al*. present the data comparing the mean post-test scores between groups. The latter approach is less sensitive to individual treatment effect, as it does not incorporate the baseline level of the outcome

parameter. For example, in the study by Sutbeyaz *et al*. the mean (SD) PImax measurements post-intervention for the IMT and the control group were 57.3 (8.6) cmH_2O and 53.9 (6.3) cmH_2O, respectively. The mean difference between the two groups was 3.4 cmH_2O, with a 95% confidence interval of –2.0 to 8.8 cmH_2O. While the mean difference favours the intervention, the confidence interval includes the value of indifference (zero), indicating that the difference did not reach statistical significance. If, however, the change scores or the difference in change scores between groups are assessed, this takes account of group differences at baseline and arguably more adequately reflects individual treatment effect. In the same example, the mean PImax at baseline was slightly lower in the IMT group (49.5 cmH_2O) than in the control group (51.0 cmH_2O), which means that the IMT group improved to a greater extent (by 7.9 cmH_2O compared to 2.9 cmH_2O in the control group) than is reflected by comparing group means post-treatment. The analysis of change scores in this instance does give a statistically significant result in favour of the intervention (mean difference 5.0 cmH_2O, 95% CI 1.2, 8.7).

In summary, the systematic reviews by Xiao *et al*. and Pollock *et al*. provide some high-level evidence that IMT in stroke leads to improvement in inspiratory muscle strength as measured by PImax, although the overall effect size can be considered modest. The reviews illustrate the paucity of evidence in the field, thus corroborating the search strategy and the findings of the present literature search and supporting the argument for further research.

1.5.3 Discussion

In the literature search conducted for this review, only four published studies of RMT in stroke were found. The paucity of published studies is corroborated by two systematic reviews of the topic. The four original research studies included in this review differ considerably in several aspects, from sample characteristics to specifics of the investigated RMT interventions. The studies also differ with respect to methodological quality. The studies by Britto *et al*. and Sutbeyaz *et al*. were randomised controlled trials with relatively high methodological quality, and a high level of confidence can be placed in their internal validity and reported results. The methodological quality of the studies by Fernandes *et al*. and Jung *et al*. could not be

adequately assessed due to lack of detail given in the publications. Table 6 gives an overview of the methodological quality of the four studies using the quality assessment criteria for randomised controlled trials suggested by Verhagen *et al*. (1998).

Despite differences between these studies, several practical aspects of research conduct specific to the intervention and the clinical population were thought to be relevant and transferable to the present studies: mode of administration of RMT; duration, frequency and intensity of training; adverse effects of training; adherence to treatment and participant retention; methods of assessing the effects of training; and reported magnitude of training effects. These aspects are summarised in Table 7 and discussed in detail in the following sections.

Table 6. Assessment of methodological quality of research studies included in this review, using the quality criteria from Verhagen *et al.* (1998).

		Britto *et al*. 2011	Sutbeyaz *et al*. 2010	Fernandes *et al*. 2007	Jung *et al*. 2014
1.	Treatment allocation				
	a) Was a method of randomisation performed?	Yes	Yes	Not stated	Yes
	b) Was the treatment allocation concealed?	Yes	Yes	Not stated	Not stated
2.	Were the groups similar at baseline regarding the most important prognostic indicators?	No	Yes	Not stated	Not stated
3.	Were the eligibility criteria specified?	Yes	Yes	Not stated	Yes
4.	Was the outcome assessor blinded?	Yes	Yes	Not stated	Not stated
5.	Was the care provider blinded?	Not stated	Not stated	Not stated	Not stated
6.	Was the patient blinded?	Yes	No	Not stated	Not stated
7.	Were point estimates and measures of variability presented for the primary outcome measures?	Yes	Yes	Yes	Yes
8.	Did the analysis include an intention-to-treat analysis?	Not stated	Yes	Not stated	Not stated

Table **7.** Details of administration of respiratory muscle training (RMT) and outcome assessment in the four original studies of RMT in stroke.

Study	Mode of delivery of RMT	Duration, frequency and intensity of training	Adverse effects of RMT	Adherence to treatment	Participant retention	Methods of assessing training effects / outcome measurements
Britto *et al*. 2011	Threshold IMT (Philips Respironics); self-training and biweekly sessions supervised by a researcher	Eight weeks, daily for five days per week, 30 minutes per session (6x5 minutes of breathing with intermittent rests of one minute duration); resistance set at 30% of PImax and adjusted biweekly by researcher	Not stated	Assessed with self-completed training diary; of a maximum of 1200 prescribed training minutes, the intervention group trained on average 1,141 minutes (range 780 to 1,560) and the control group 1,160 minutes (range 600 to 1,860)	Out of 21 participants two dropped out due to lack of time (intervention group) and one due to hypertension (control group); 18 participants completed the study	Maximal inspiratory mouth pressure (PImax) Inspiratory muscle endurance (incremental threshold loading method) Functional performance (incremental cycle ergometer test, maximum activity score of the Human Activity Profile) Health-related quality of life (Nottingham Health Profile)
Sutbeyaz *et al*. 2010	Threshold IMT (Philips Respironics); level of supervision or assistance provided by health staff not stated	Six weeks, twice daily for six days per week, 15 minutes per session; resistance adjusted to 40% of PImax initially, then gradually increased to 60% of PImax; number of breaths per session not stated	Not stated	Not stated	45 out of 45 participants completed the study	Lung function testing (VC, FVC, FEV_1, FEF_{25-75}, PEF, MVV) Maximal mouth pressures (PImax, PEmax) Incremental hand crank ergometer test (VO_2peak, HRpeak, $V_E$peak, SaO_2, VD/VTpeak, power output, perceived dyspnoea) Functional status (Barthel Index, Functional Ambulation Categories) Health-related quality of life (SF-36)

Table 7. continued

Fernandes *et al*. 2007	Threshold PEP (Philips Respironics); level of supervision or assistance provided by health staff not stated	Five days; daily 5x10 breaths with intermittent rests of one minute duration; resistance set at 40% of PEmax	Not stated	Not stated	Not stated	Maximal mouth pressures (PImax, PEmax) Peak expiratory flow (PEF)
Jung *et al*. 2014	Threshold IMT (Philips Respironics); level of supervision or assistance provided by health staff not stated	Four weeks, 20 minutes per day three times per week; resistance set at 30% of PImax	Not stated	Not stated	Not stated	Forced spirometry (FVC, FEV_1, FEF_{25-75}, PEF) Diaphragm thickening ratio

FEF_{25-75}, forced expiratory flow between 25% and 75% of the expired volume; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; HRpeak, peak heart rate; IMT, inspiratory muscle training; MVV, maximum voluntary ventilation; PEF, peak expiratory flow; PEmax, maximal expiratory mouth pressure; PEP, positive expiratory pressure; PImax, maximal inspiratory mouth pressure; RMT, respiratory muscle training; SaO_2, arterial oxygen saturation; SF-36, short form 36; VC, vital capacity; VD/VTpeak, peak dead space – tidal volume ratio; $V_E$peak, peak minute ventilation; VO_2peak, peak oxygen consumption

1.5.3.1 Mode of administration of respiratory muscle training

In all four studies by Britto *et al*., Sutbeyaz *et al*., Fernandes *et al*. and Jung *et al*., the threshold loading technique was used to administer RMT. Commercially available devices (Threshold IMT and Threshold PEP, Philips Respironics, Andover, MA) were used for inspiratory and expiratory training, respectively. No rationale was given for the choice of RMT technique. However, threshold loading is generally described as a more practical and controllable training method compared with alternative techniques (Reid & Samrai, 1995).

Only Britto *et al*. described the level of training supervision provided in their study. Participants trained independently at their homes, and biweekly sessions with a researcher were held to observe training technique and adjust threshold pressures. The level of training supervision or assistance given is not described in the papers by Sutbeyaz *et al*., Fernandes *et al*. and Jung *et al*., although it may be assumed that training sessions were overseen by research or health care staff where participants were admitted as inpatients (*i.e.* studies by Sutbeyaz *et al*. and Fernandes *et al*.). The provision of supervision for training interventions in research requires careful consideration. On the one hand, a high level of supervision and personal coaching are likely to increase training fidelity and quality, and thereby increase confidence in the internal validity of the research. On the other hand, the level of staff resources utilised to provide training supervision in a research context also impacts on the external validity and transferability of the research. In particular, a problem may arise if it is not possible to match the level of supervision that was provided during the conduct of a research study when attempting to implement a training intervention in real-life clinical practice. In this respect, a pragmatic research design, in which staffing resources from real-life clinical practice are replicated, holds greater external validity.

1.5.3.2 Duration, frequency and intensity of training

Participants in the study by Britto *et al*. trained for a period of eight weeks for five days per week. Each training session lasted for 30 minutes, whereby participants breathed through the threshold resistance device for 6x5 minutes with intermittent rests of one minute duration. Sutbeyaz *et al*. delivered training over a period of six weeks, twice daily for six days per week. Participants trained for 15 minutes per session. In the study by Fernandes *et al*. participants trained daily for five days and took 5x10 breaths with intermittent rests of one minute duration. In the study by Jung *et al*., training was delivered in three weekly sessions of 20 minutes duration. Threshold resistance was set at 30% of PImax in the studies by Britto *et al*. and Jung *et al*. Participants in the study by Sutbeyaz *et al*. started training at 40% of PImax, and resistance was gradually increased over the duration of the intervention to 60% of PImax. In the study by Fernandes *et al*. threshold resistance was set at 40% of PEmax.

In the seven randomised controlled trials of RMT in neurological populations other than stroke, which were included in the systematic review by Pollock *et al*., the following range of training parameters was applied: duration of training from eight to 12 weeks; frequency of training from three times per week to three times per day; session duration from 10 minutes to 30 minutes per session, or sets of 3x10 to 3x15 breaths per session; and resistance at 20% to 60% of PImax or PEmax. In one study resistance was set at the highest level that could be maintained throughout the session. These RMT parameters lie within the wider range of training protocols used in research in cardio-respiratory populations (Geddes *et al*. 2008, Weiner & McConnell 2005, Reid & Samrai 1995). Overall, while there is some variability in the delivery of RMT across clinical studies, these training protocols provide a general framework for future study designs. Due to the differences in study populations, study settings, and differences in the clinical rationales for providing RMT, an attempt to relate specific training protocols to optimum treatment effect may be of limited value.

1.5.3.3 Adverse effects of training

Adverse effects relating to RMT are not commented on in the publications by Britto *et al*., Sutbeyaz *et al*., Fernandes *et al*. and Jung *et al*. The safety and potential adverse effects of RMT find little discussion in the wider literature. For example, the recent guidelines for the physiotherapy management of the adult, medical, spontaneously breathing patient (Bott *et al*. 2009) give evidence-based recommendations on the use of RMT in different clinical groups without raising any safety concerns. The lack of reported adverse effects of RMT may be taken as evidence of the relative safety of the intervention, in particular IMT, which has been researched more extensively than expiratory muscle training (EMT) and in a range of clinical groups. EMT has been investigated less, and Weiner and McConnell (2005) discuss potential safety concerns of expiratory muscle training, suggesting that increased intra-thoracic pressure may have a negative impact on cardio-vascular function. However, no adverse events were reported in two studies of EMT by their group, which included a total of 58 patients with chronic obstructive pulmonary disease (COPD). Also, other clinical studies of EMT have not reported any safety concerns (Kim & Sapienza, 2005, Pitts *et al*. 2005, Gosselink *et al*. 2000, Smeltzer *et al*. 1996).

1.5.3.4 Participant retention and adherence to treatment

Britto *et al*. and Sutbeyaz *et al*. give a detailed description of the participant flow through their study. Of 21 participants who entered the trial by Britto *et al*., two discontinued in the intervention group due to insufficient time, and one dropped out of the control group due to hypertension. In the study by Sutbeyaz *et al*., all 45 participants (15 per group) completed the trial. Fernandes *et al*. and Jung *et al*. do not comment on participant retention in their studies, although it may be inferred from their paper that all participants completed the study.

Sutbeyaz *et al*., Fernandes *et al*. and Jung *et al*. do not comment on adherence to treatment or treatment acceptability to participants. It may be assumed that in these studies participants

were supervised by health staff when carrying out the intervention and that therefore adherence to treatment was unproblematic. In the study by Britto *et al*., participants completed training diaries, which were used to assess treatment adherence. The prescribed amount of respiratory training was a total of 1,200 minutes over eight weeks. Mean adherence was 1,141 minutes (range 780 to 1,560) in the intervention group and 1,159 minutes (range 600 to 1,860) in the control group. While the average training time approximates the prescribed intensity of training, the spread ranges from half to about 50% more than the prescribed training time.

1.5.3.5 Methods of assessing the effects of training

The studies included in this review differed with respect to research hypotheses, clinical rationales and methods of assessing the effects of RMT. The studies by Britto *et al*. and Sutbeyaz *et al*. investigated RMT in stroke with respect to its impact on general cardiovascular fitness and the further implications on daily functioning and quality of life. The study by Fernandes *et al*. and Jung *et al*. focussed solely on the effect of RMT on physiological respiratory parameters. An overview of the outcome measurements undertaken in these studies is given in Table 7. The most commonly used measure was maximal mouth pressure (PImax, PEmax). Other tests included: various physiological cardio-respiratory tests such as forced spirometry; functional measures such as the Barthel Index; and measures of health-related quality of life such as the Nottingham Health Profile. None of the studies examined cough or clinical respiratory outcomes.

1.5.3.6 Reported magnitude of training effects and clinically relevant improvement

The four studies by Britto *et al*., Sutbeyaz *et al*., Fernandes *et al*. and Jung *et al*. all reported positive effects of RMT. Insufficient information is presented in the papers by Fernandes *et al*. and Jung *et al*. to ascertain the internal validity of their study, and their results should therefore be interpreted with caution. Britto *et al*. and Sutbeyaz *et al*. were able to establish several statistically significant training effects of RMT; however, the authors offer little discussion of the

clinical relevance of these findings. Notably, all positive effects were found in physiological measures, as opposed to measures of functional performance or wellbeing. It may be argued that the value of physiological improvement is uncertain, unless it can be linked to concrete benefit for the individual's wellbeing and their ability to function in everyday life. This link can be demonstrated through according outcome measures. In the studies by Britto *et al*. and Sutbeyaz *et al*. those outcome measures that relate directly to improvement in everyday life and general wellbeing, *i.e.* measures of function and quality of life, did not show any changes.

Alternatively, the benefit of improvement in physiological outcomes may be established by comparison with norm values for healthy subjects, as these may represent a desired treatment goal. The effect size for PImax, for example, in the study by Britto *et al*. amounted to a mean (95% CI) increase of 34.4 (13.6, 55.2) cmH_2O from a mean PImax at baseline of 67.8 cmH_2O. In the study by Sutbeyaz *et al*. the mean (95% CI) improvement was 7.9 (4.2, 11.5) cmH_2O from a mean PImax at baseline of 49.5 cmH_2O. In both studies, groups consisted of equal numbers of male and female participants. Comparing these values with PImax norm values in healthy subjects (105-129 cmH_2O in men; 70-98 cmH_2O in women) (American Thoracic Society (ATS) & European Respiratory Society (ERS), 2002, p. 532), it could be suggested that in the study by Britto *et al*. RMT potentially restored PImax to near normal levels (taking into consideration the wide 95% confidence interval). In contrast, in the study by Sutbeyaz *et al*. RMT lead to a small improvement towards healthy norm values.

1.5.3.7 Considerations informing the present research design

To summarise, from the considerations discussed in sections 1.5.3.1 through 1.5.3.6, the following decisions were made with respect to the design and conduct of the present research:

Regarding the level of supervision and assistance provided during the training implementation, a pragmatic design, replicating staffing resources from real-life clinical practice, was selected to maximise generalisability to UK National Health Service (NHS) clinical settings.

The RMT protocol chosen for the present study (training duration of four weeks; daily sessions of 5x10 breaths with intermittent one minute rests; resistance set at 50% of PImax or PEmax, respectively) had one of the shortest durations, and frequency and intensity parameters within the higher range compared to other protocols described in the literature. Judging from previous research, this training protocol could reasonably be expected to induce a training effect; while time afforded to training, and therefore participant burden, was kept to a minimum. Also, these training parameters mirrored the physiological purpose of the intervention, *i.e.* improving cough, which occurs in short bursts of high intensity, as opposed to *e.g*. endurance-related performance, which may require lower intensity training over longer periods of time.

Regarding adverse effects of RMT, the present research was the first to investigate both in- and expiratory training early after acute stroke, as participants were recruited within two weeks of stroke onset. While from published research there is no indication that RMT may cause harm in this population, the introduction of a new intervention in the acute stage of a clinical condition warrants careful monitoring of the safety of training. Accordingly, the present research included monitoring of vital parameters (blood pressure, heart rate, blood oxygen saturation) and subjective adverse clinical signs before and after training at baseline and in subsequent weekly sessions with the investigator.

With respect to study outcomes, in contrast to previous studies, the present research focused on peak cough flow as the primary physiological outcome measure, and also included maximal mouth pressures and incidence of pneumonia as secondary endpoints. The present research was likely the first to investigate the effect of RMT on cough flow in stroke survivors. A small number of RMT studies in clinical populations other than stroke have included cough parameters as outcomes. Gosselink *et al*. (2000) conducted a randomised controlled trial comparing EMT with unsupervised unspecific breathing exercises in severely impaired patients with multiple sclerosis. Cough was assessed using the Pulmonary Index (PI), a clinical measure using a combination of objective and subjective criteria to quantify cough efficacy. Their study showed statistically significant improvement in the PI in the EMT group. A study by Salem *et al*.

(2004, cited in Kim & Sapienza, 2005) showed improved expiratory flow in voluntary cough after EMT in patients with Parkinson's disease, while a cohort study by Chiara *et al*. (2006) failed to demonstrate improvements in cough flow after EMT in multiple sclerosis patients. More recently, Kim *et al*. (2009) conducted a cohort study of healthy sedentary older adults, which showed a statistically significant increase in expiratory flow during capsaicin-induced reflex cough after EMT. In contrast, in a cohort study of individuals with Parkinson's disease by Pitts *et al*. (2009) voluntary expiratory cough flow remained unchanged after EMT. The hypothesis that RMT may improve cough effectiveness is discussed frequently in relevant publications. However, little published evidence is available, and this supports the argument for further research.

1.6 Summary

In summary, this introduction described the context for the present research and gave a justification for its clinical relevance. From a narrative review of the literature, it was established that pneumonia after stroke remains a current clinical concern, with in-hospital incidence rates between 6% and 16% internationally. Patients who develop pneumonia after stroke have a two- to six-fold increase in risk of death, are more likely to have poor rehabilitation outcomes and on average require higher levels of hospital and community care.

It was shown that one of the most consistently reported risk factors for pneumonia after stroke is the presence of swallowing difficulty; and that the most widely applied prevention strategy is the routine screening of stroke patients for swallowing difficulty coupled with the implementation of dysphagia management strategies. Different novel strategies for reducing pneumonia incidence after stroke have been and are currently being researched. The present research took an original approach in investigating respiratory muscle strengthening as a non-pharmacological intervention, with the aim to improve cough effectiveness and increase airway protection in acute stroke.

The rationale for this novel approach was provided, first, by clinical evidence, which demonstrates that increased pneumonia risk after stroke is associated with aspiration and cough impairment; and second, by physiological evidence, which shows that there is significant impairment of cough in acute stroke, and that this impairment can be linked to respiratory muscle weakness. Thus, a respiratory muscle strengthening intervention may prove useful in improving cough effectiveness, increasing airway protection, and ultimately reducing pneumonia risk in acute stroke.

RMT using the pressure threshold loading technique was identified as the most appropriate intervention. This intervention is supported by an extensive evidence base derived from healthy and athletic populations and a fair evidence base from primarily cardio-respiratory clinical populations. A systematic review of the literature was conducted to establish the evidence base for RMT in stroke, and it was found that little research is available. Two randomised controlled trials of good methodological quality have demonstrated that RMT in chronic and subacute stroke survivors leads to improvements in physiological parameters of respiratory muscle strength, lung function and endurance. A meta-analysis of randomised controlled trials in different neurological conditions showed a modest strengthening effect of RMT on inspiratory muscles. No previous studies of RMT in stroke have included patients in the acute phase of stroke; investigated RMT with the aim to improve cough effectiveness; or investigated RMT for the prevention of pneumonia. Based on the findings of this literature search, the present research was the first of its kind and provided valuable evidence in a little researched field.

Chapter 2 Aims and objectives

The overarching aim of the present studies was to investigate the merit of respiratory muscle training as an intervention for the reduction of pneumonia risk during the first weeks after stroke. The specific aims and objectives relating to each thesis chapter are as follows:

Chapter 3 describes a series of validation experiments of three respiratory assessment methods: forced spirometry, measurement of maximal mouth pressures, and cough flow measurement. The aim was to evaluate measurement variability when applying these assessment procedures in novel patient groups and settings different from those in which they have previously been validated. The purpose was to allow an interpretation of clinical findings against the estimated magnitude of measurement error that may be expected with respect to the particular equipment and testing procedures and in this clinical population. The specific objectives were:

- To describe instrument repeatability
- To describe test-retest reliability in the absence of clinical change, both in healthy subjects and in acute stroke patients
- To describe instrument performance properties with specific relevance to the cough flow measurement system (linearity and dynamic response)
- To determine the minimal detectable difference for the main outcome parameters of interest (maximal expiratory and inspiratory mouth pressure, peak expiratory cough flow of voluntary and reflex cough)

Chapter 4 summarises a series of validation experiments with the aim to compare the accuracy in measuring peak cough flow between portable flow measuring devices and the flow measurement system used in the present research. The purpose was to investigate whether the flow measurement system used in the present studies, which provided physiologically detailed measurements but consisted of an elaborate and expensive equipment setup, could potentially

be substituted with convenient and less expensive hand-held clinical flow measurement devices. The specific objectives were:

- To describe the accuracy of devices when measuring peak expiratory cough flow
- To explore potential sources of the observed inaccuracy.

Chapter 5 gives an account of the main clinical study of this thesis. The aim of this pilot study was to investigate a respiratory muscle strengthening programme in the first weeks after stroke, providing estimates on its magnitude of effect, safety, acceptability and feasibility, and informing about the value and design of a large clinical trial. The specific objectives were:

- To determine the magnitude of effect of respiratory muscle training on cough generation, respiratory muscle strength, and incidence of pneumonia
- To explore the training duration, frequency and intensity required to achieve improvement in cough flow rate and inspiratory and expiratory muscle strength
- To evaluate patient participation, acceptability of study procedures to participants, and concordance with training protocol
- To describe safety parameters and potential adverse effects of respiratory muscle training in this patient group
- To describe characteristics of those patients most likely to gain from the intervention
- To determine the relevance and feasibility of delivering respiratory muscle training to acute stroke patients in UK National Health Service (NHS) settings.

Chapter 6 describes a secondary exploratory analysis of data from the main clinical study. The aim was to use these data to describe the relevance of cough as a mediating parameter in the relationship between swallowing impairment and pneumonia in acute stroke. Little published data is available about this particular interaction. The specific objectives were:

- To explore pneumonia risk according to parameters of respiratory function, respiratory muscle strength and cough function
- To quantify the interaction between peak cough flow, aspiration risk and pneumonia risk.

Chapter 7 presents data from cough frequency measurements using an automated cough counting device (Leicester Cough Monitor) in a subgroup of participants. The aim was to validate the device and to explore longitudinal measurements of cough frequency in a cohort of acute stroke patients. The specific objectives were:

- To validate the Leicester Cough Monitor as a method of cough frequency measurement in acute stroke
- To describe cough frequency over 24-hour periods at baseline, and at one week, four weeks and twelve weeks after baseline
- To compare the observed cough frequency in stroke survivors with normative values
- To explore relationships between cough frequency and other relevant patient characteristics, including the severity of stroke impairment, presence of swallowing impairment, lung function and cough intensity.

Chapter 3 Measurement properties relating to three respiratory assessment methods

3.1 Introduction

This chapter describes a series of experiments for the validation of three respiratory assessment methods applied in subsequent studies: forced spirometry, measurement of maximal mouth pressures and cough flow measurement. Equipment and testing procedures for these assessments are described. These validation experiments include laboratory bench tests and tests with healthy volunteers and stroke patients. The primary purpose of these validation studies was to evaluate the level of measurement error inherent in the assessment procedures, which subsequently informed the analysis and interpretation of clinical data.

Measurement error, uncertainty or variability relates to the fact that repeated measurements of the same quantity may yield different results. The magnitude of the discrepancy between these results indicates the precision or reliability of the measurement. The wider the discrepancy, the less precise is the measurement, and vice versa (Taylor 1997, pp. 3-11). Variability in measurements of physiological parameters in human subjects can generally be related to four aspects of the measurement process: the measurement instrument; a single observer (intra-rater); different observers (inter-rater); and the subject (intra-subject) (Domholdt 2005, pp. 243-274).

One important consideration with respect to the assessments evaluated here is that these are measurements of volitional respiratory manoeuvres, for which the test subject is asked to produce maximal efforts. The aspect of subjective motivation and effort therefore needs to be considered as a relevant source of measurement error (unlike when measuring a quantity that does not require active engagement from the test subject). A second consideration is that in order to increase the assessor's confidence that a maximal effort has been exerted, it is

convention to ask the test subject to repeat the manoeuvre several times. The principle is that consecutive efforts can be compared against each other, judging the relative influence of familiarisation with the task (improving performances with increasing number of repetitions) and fatigue (worsening performances with increasing number of repetitions). In practice this means that in a single sitting, the volitional test manoeuvre should be repeated several times to allow the subject familiarisation with the technique; but that the maximal number of repetitions should be limited to prevent fatigue and overexertion. A strategy that is commonly applied in practice is to observe whether a number of measurements within a certain range of the maximum can be achieved, which is taken as an indication that the subject's maximal volitional effort has been reached. As an example, the international guidelines for the standardisation of forced spirometry (Miller *et al*. 2005, p. 326) state that three manoeuvres within a certain margin of the best effort (largest forced vital capacity and forced expiratory volume in one second) need to be achieved, but that no more than eight attempts should be performed altogether in one sitting.

3.2 Aims and objectives

The aim of this study was to evaluate the measurement variability for forced spirometry, measurement of maximal mouth pressures and cough flow measurement when applying the same assessment procedures as in subsequent clinical studies.

The objectives were to:

- Describe instrument repeatability
- Describe test-retest reliability in the absence of clinical change, both in healthy subjects and in acute stroke patients
- Describe instrument performance properties with specific relevance to the cough flow measurement system (linearity and dynamic response)
- Determine the minimal detectable difference for the outcome parameters of interest (maximal expiratory and inspiratory mouth pressure, peak expiratory cough flow of voluntary and reflex cough)

3.3 Methods

3.3.1 Study design

To examine instrument repeatability, a series of bench tests were conducted at the respiratory physiology laboratory at the Department of Respiratory Medicine and Allergy, School of Medicine, King's College London. Relevant inputs (volume, pressure, flow) of a known and consistent quantity were repeatedly delivered and measured with the instruments under test, allowing an assessment of the consistency of repeated measurements.

To examine test-retest reliability, healthy subjects and stroke patients performed the tests following the same sequence and procedures as in the subsequent clinical studies. One investigator conducted all assessments. All tests were repeated within the time period of two hours. It was assumed that no physiological change had occurred in test subjects within this time window and that the variability observed could be interpreted as measurement variability in the absence of change.

While the evaluation of instrument repeatability informs about the extent of measurement error that can be ascribed to the performance of the measurement instrument alone, test-retest reliability gives an estimate of measurement variability in the absence of change, incorporating all possible sources of measurement error, *i.e.* instrument repeatability, intra-rater variability and intra-subject variability. As in the subsequent clinical studies all measurements were performed by one investigator, inter-rater variability was not relevant for the present study.

3.3.2 Participants

Eleven healthy volunteers (mean (SD) age 40 (14) years, six men) were recruited from amongst staff and students at King's College London. Excluded were individuals with a medical history of respiratory disease or conditions affecting larynx structure and function. Volunteers had to be comfortable coughing repeatedly and performing repeated maximal respiratory manoeuvres over two hours. Ethical approval for the recruitment of healthy volunteers was granted by the Psychiatry, Nursing and Midwifery Research Ethics Committee at King's College London, UK (study reference PNM/12/13-143). All healthy volunteers gave written informed consent.

Six acute stroke patients (mean (SD) age 51 (16) years, four men) took part in eleven testing sessions, giving data for eleven test-retest reliability assessment sessions. Patients were recruited from the hyper-acute stroke unit at King's College Hospital, London. Patients were included if they had moderate to severe level of stroke impairment (National Institutes of Health Stroke Scale (NIHSS; International Electronic Education Network 2010) score 5-25) and were able to follow instructions and perform testing procedures. Patients were excluded if they had a history of respiratory disease, recent cardiac events, or neurological conditions other than stroke. Patients took part in assessment sessions between four and twelve weeks after stroke onset. Ethical approval for the recruitment of stroke patients was granted by the UK National Research Ethics Service (NRES) (Wandsworth Research Ethics Committee, study reference 10/H0803/32). All stroke subjects gave written informed consent.

3.3.3 Respiratory assessments

3.3.3.1 Forced spirometry

3.3.3.1.1 Equipment

A turbine-based hand-held portable spirometer (SpiroUSB, CareFusion, San Diego, California) connecting to a laptop with accompanying software was used. For infection control purposes, a disposable bacterial filter was used (Spiroguard Standard, Air Safety Medical, Morecambe, England). A flanged mouth piece (Rubber Flanged Mouthpiece MTH6400, CareFusion, San Diego, California) was used to create an optimal mouth seal in the presence of oro-facial weakness. A nose clip (Nose Clip, Air Safety Medical, Morecambe, England) was used to avoid loss of airflow through the nostrils.

3.3.3.1.2 Testing procedure

Forced spirometry was conducted according to international guidelines (Miller *et al.* 2005). Test subjects were seated comfortably in a chair or positioned sitting up in the hospital bed. The investigator explained the procedure, and demonstrated the correct technique for a forced expiratory manoeuvre (without equipment). If required, the investigator assisted participants in holding the spirometer and achieving adequate positioning of the mouth piece. Test subjects performed at least three and up to eight forced expiratory manoeuvres. The investigator gave verbal instructions and enthusiastic encouragement during each manoeuvre, and verbal feedback on technique after each manoeuvre. The test was concluded when quality requirements were met (three acceptable manoeuvres, with the two largest measurements of forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) within 0.15 L, or within 0.1 L for FVC<1.0 L) (Miller *et* al. 2005). From the three acceptable manoeuvres with the largest values, the highest FVC, FEV_1 and peak expiratory flow (PEF) were recorded and used for analysis, whereby individual values could be taken from different manoeuvres. On occasion, it was not possible to achieve quality requirements within eight attempts. This was mostly due to

inconsistent performance and fatigue in acute stroke patients. In these cases, FVC, FEV_1 and PEF values were selected from the three manoeuvres with the highest FVC.

3.3.3.1.3 Quality control

Volume accuracy of the spirometer was evaluated on each day of testing with a volume calibration check as per manufacturer's guidelines and as recommended by international guidelines (Miller *et al*. 2005, p. 323). Using a three-litre calibration syringe (Series 5530, Hans Rudolph Inc, Kansas City, Minnesota, USA), three litre volumes were delivered through the spirometer at three levels of flow (0-54 L/min, 96-270 L/min and 420-720 L/min), to ensure that the measured volume was within the acceptable 3.5% error margin.

3.3.3.2 Maximal mouth pressure measurements

3.3.3.2.1 Equipment

A portable device (MicroRPM, CareFusion, San Diego, California) was used with a disposable bacterial filter (Mouth Pressure Bacterial Filters FIL6050, CareFusion, San Diego, California), a flanged mouth piece (Rubber Flanged Mouthpiece MTH6400, CareFusion, San Diego, California), and a nose clip (Nose Clip, Air Safety Medical, Morecambe, England).

3.3.3.2.2 Testing procedure

Measurements of maximal respiratory mouth pressures (PImax, PEmax) were conducted according to international guidelines (American Thoracic Society (ATS) & European Respiratory Society (ERS) 2002, pp. 531-533). Test subjects were seated comfortably in a chair or positioned sitting up in the hospital bed. The investigator explained the procedure, and demonstrated the correct technique (without equipment). For the assessment of PEmax, the subject was asked to breathe in to total lung capacity and maintain a maximal forced expiration

into the MicroRPM device for three seconds. For the measurement of PImax, the subject was asked to breathe out to residual volume and maintain a maximal forced inspiration through the MicroRPM device for three seconds. The investigator gave verbal instructions and enthusiastic encouragement during each manoeuvre, and verbal feedback on technique after each manoeuvre, paying particular attention to adequate lip seal around the mouth piece. A minimum of five and up to ten attempts were performed for PEmax and PImax, respectively, until the three highest measurements were within 20% of the maximum. The maximum value of the three highest measurements was recorded for analysis. On occasion, it was not possible to achieve three highest measurements within 20% of the maximum. This was mostly due to inconsistent performance and fatigue in acute stroke patients. In these cases, the highest measurement was taken for analysis.

3.3.3.2.3 Quality control

Pressure accuracy of the MicroRPM device was confirmed weekly by comparison against a digital manometer. A three-way airtight tubing system was used to connect the MicroRPM device with the digital manometer (C9553 Pressure Meter, Comark, Norwich, England) and an inflation device (Encore 26 Inflator, Boston Scientific, Marlborough, Massachusetts, USA). Positive and negative pressure was applied to 200 and -200 cmH_2O, respectively, as measured by the digital manometer. The reading given by the MicroRPM device was compared to the digital manometer reading, to ensure that the MicroRPM measured pressure within the acceptable error margin.

3.3.3.3 Cough flow measurements

3.3.3.3.1 Equipment

Cough flow measurements were conducted with a calibrated pneumotachograph system (Singh *et al.* 1994). An on-site and an off-site measurement system were used. The on-site system was used for measurements at the primary study site, King's College Hospital, London. The off-site

system was used for measurements outside the primary study site, for example at the neighbouring stroke rehabilitation unit or at participants' homes. The following components were used for the cough testing equipment when cough testing was conducted on site: face mask (Adult Face Mask, 8940 Series, Hans Rudolph Inc, Kansas City, Missouri) with connector, head straps and clips; Fleisch-type pneumotachograph (internal diameter (ID) 4.4 cm, length 6.0 cm, PK Morgan Ltd, Rainham, England); connecting tubing (ID 3.0 mm, length 155.0 cm); pressure transducer (MP45-14-871 Low Range Differential Pressure Transducer, range ± 2 cmH_2O, Validyne Engineering, Northridge, California); demodulator (CD15 Sine Wave Demodulator, CD15-C-1-A-1, Validyne Engineering, Northridge, California); analog-to-digital converter (PowerLab/16SP, ADInstruments Ltd, Oxford, England); and laptop running data acquisition software (LabChart 7 Pro, v7.2.2, 24 June 2011, ADInstruments Ltd, Oxford, England) (Figure 2).

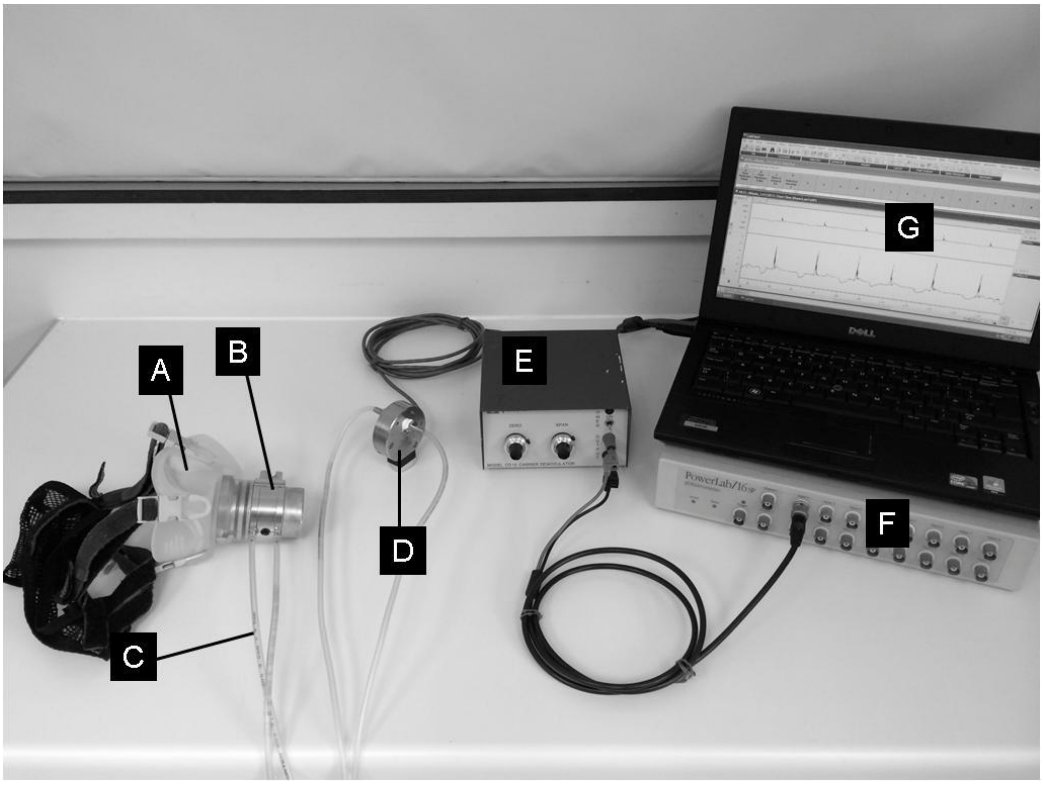


Figure 2. On-site system for cough flow testing. A, face mask; B, pneumotachograph; C, connecting tubing; D, pressure transducer; E, demodulator; F, analog-to-digital converter; G, laptop and software.

When cough testing was conducted off-site, an alternative setup was used, which had better practicality and portability. In the portable setup, a different analog-to-digital converter (NI BNC-2110, National Instruments, Newbury, England) and laptop with software (LabView 5.1, Copyright 2000, National Instruments, Newbury, England) were used (Figure 3).

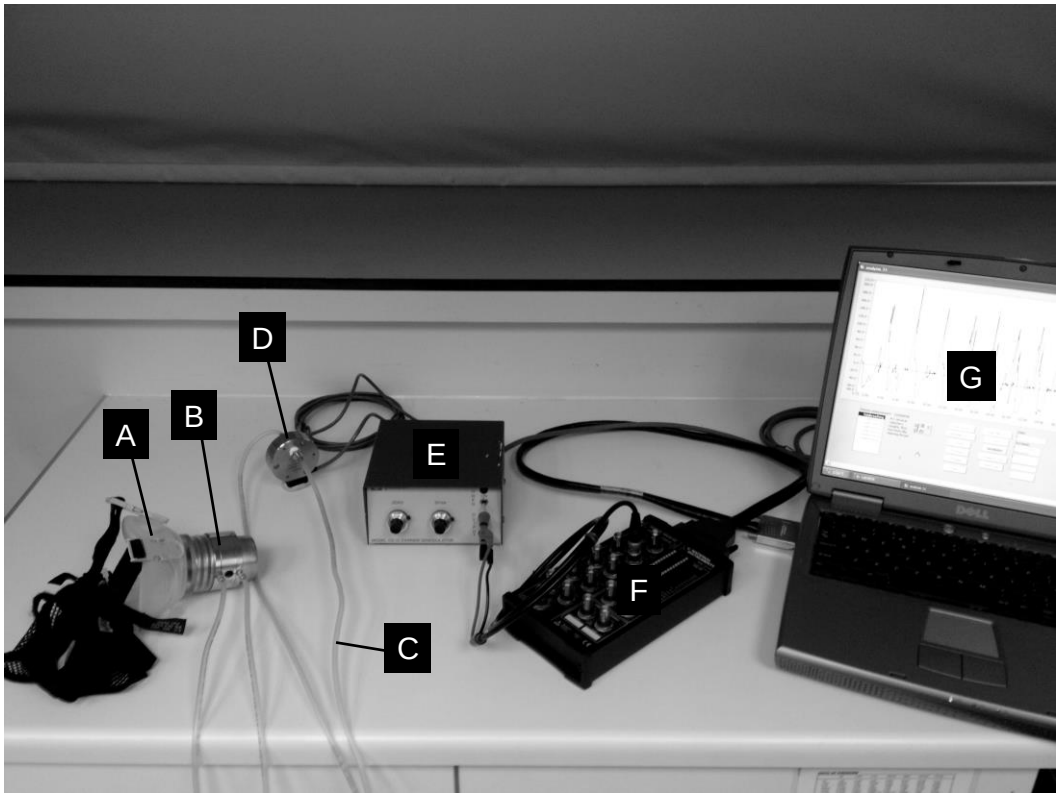


Figure 3. Off-site system for cough flow testing. A, face mask; B, pneumotachograph; C, connecting tubing; D, pressure transducer; E, demodulator; F, analog-to-digital converter; G, laptop and software

Analog-to-digital sampling rate for both systems was 2,000 Hz. The pneumotachograph systems were calibrated before each testing session by two-point flow calibration with a rotameter (InFlux OF1"S, 60-600 L/min flow, Techniquip Ltd, Taunton, England) using a reference flow of 500 L/min (Figure 4).

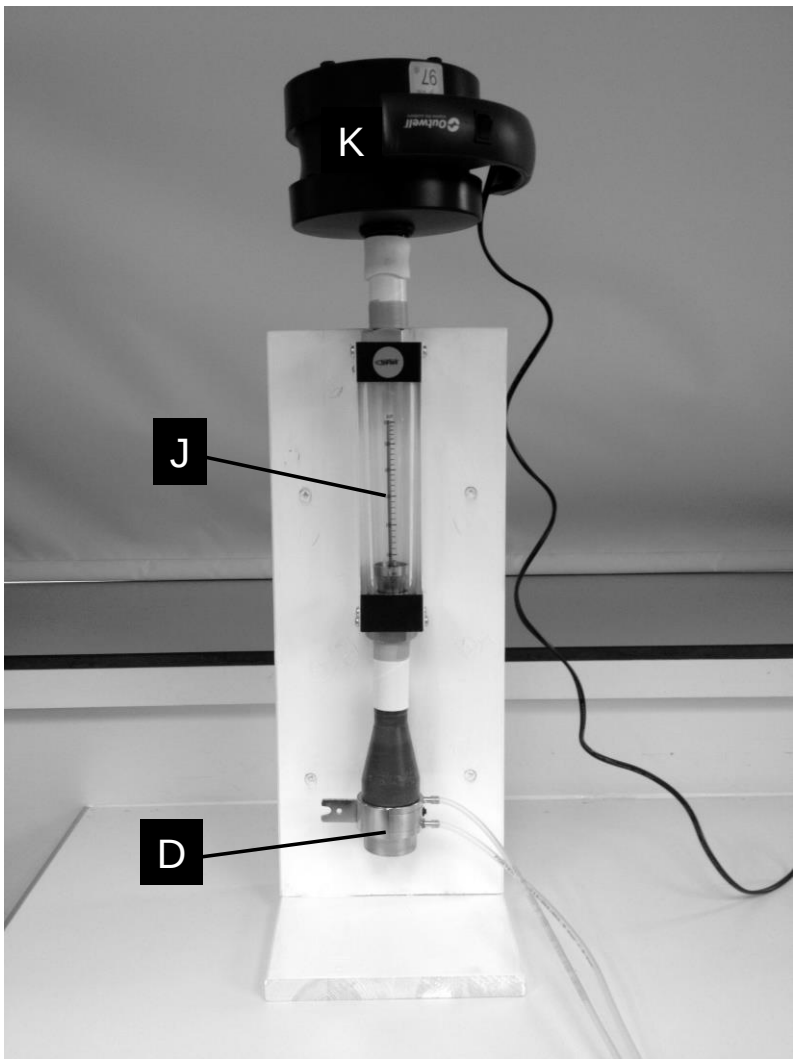


Figure 4. Two-point flow calibration at 500 L/min reference flow. D, pneumotachograph; J, rotameter (InFlux); K, flow generating device (Outwell Thunder High Performance Pump)

3.3.3.3.2 Testing procedure

Voluntary and reflex coughs were assessed. Test subjects were seated comfortably in a chair or positioned sitting up in the hospital bed. For voluntary coughs, participants made up to 15 maximal cough efforts into a tight-fitting face mask, until five coughs with peak expiratory cough flow (PECF) within 5% of the highest reading were recorded. The investigator's verbal instruction was to 'take a deep breath in and give a strong cough'. The investigator demonstrated a strong voluntary cough (without equipment). During recording, the investigator evaluated the quality of the subject's cough sounds and cough flow traces. Inadequate

attempts, such as forceful clearing of the throat or forced expiratory manoeuvres without glottis closure, were noted.

To elicit reflex coughs, solutions of capsaicin in escalating concentrations (0.49 to 1,000 μMol/L) were nebulised with an ultrasonic nebuliser (UltraNeb U3000, DeVilbiss Healthcare Ltd, Tipton, England). The nebuliser chamber was connected downstream to the pneumotachograph via a non-rebreathing valve (Two-Way T-Shape Non-Rebreathing Valve Series 2700, Hans Rudolph Inc, Shawnee, Kansas) and corrugated tubing (Limblite AMCA 1400/1, ID 2.2 cm, length 200.0 cm, Armstrong Medical Ltd, Coleraine, Northern Ireland). To contain the nebulised capsaicin within the system, a filter (Barr-vent S filter 300 400 000, Medisize bv, Hillegom, Netherlands) was connected to the exhalation port of the non-rebreathing valve. Escalating concentrations of nebulised capsaicin were introduced into the face mask for one minute at a time, until the threshold was reached at which at least five bouts of reflex coughing were triggered. Subjects were instructed to take deep breaths in and out, and to only cough if stimulated by the irritant. Of note, nebulised capsaicin was used for the sole purpose of triggering involuntary coughs, and the concentration at which coughs were elicited cannot reliably be taken as a measure of subjects' reflex cough sensitivity, as this method of eliciting reflex coughs does not meet the methodological requirements of inhalation cough challenges for assessment of reflex cough sensitivity (Morice *et al*. 2007).

Data was extracted from the five voluntary and five reflex cough flow traces with the highest peak expiratory flow values. A sample flow trace is shown in Figure 5. The values for peak inspiratory cough flow (PICF) and PECF were obtained from the minimum and maximum on the respective portions of the flow trace. Cough volume inspired (CVI) and cough volume expired (CVE) were obtained by integrating the area under the curve of the inspiratory and expulsive phase portions of the flow curve. Glottis compression time (GCT) was defined as the section between inspiratory and expulsive cough phase where the flow trace approximates zero. Rise time was defined as the time from the beginning of the expulsive phase to the point of PECF. Cough volume acceleration (CVAC) was calculated as PECF/rise time (L/s/s). The highest

values for each of these parameters were recorded and used for analysis, whereby values could be taken from different cough manoeuvres.

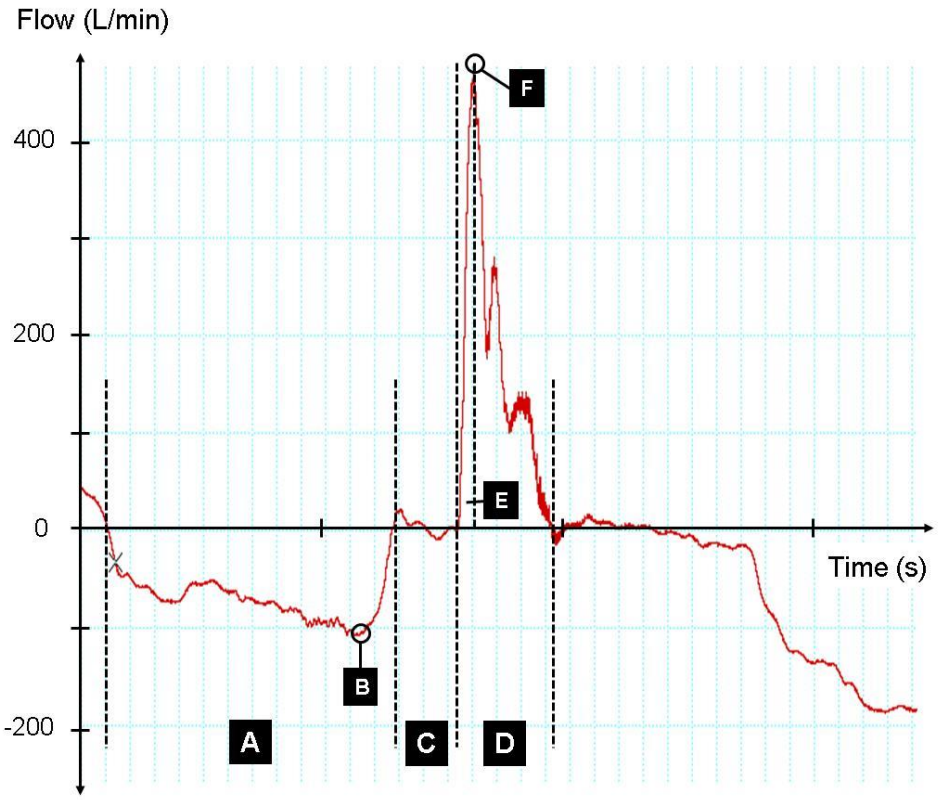


Figure 5. Flow time trace of a voluntary cough. A, inspiratory phase; B, peak inspiratory cough flow; C, glottis compression phase; D, expulsive phase; E, rise time; F, peak expiratory cough flow.

Measurements of gas volume and flow are affected by temperature, pressure and water vapour saturation. Within the lungs, air temperature and water vapour saturation increase in comparison to ambient conditions. This leads to a discrepancy between inspiratory and expiratory volume and flow measurements. Inspiratory measurements are therefore corrected to lung conditions, using body temperature, pressure, water vapour saturated (BTPS) correction factors. (Miller *et al*. 2005, pp. 332-333, American Thoracic Society (ATS) 1995, p. 1115). PICF and CVI were adjusted by multiplication with BTPS correction factors according to ambient temperature (Table 8). Ambient temperature was measured using a digital thermometer (Digital Thermo, Russell Scientific Instruments Ltd, Dereham, England).

Table 8. Ambient temperature, pressure, water vapour saturated (ATPS) to body temperature, pressure, water vapour saturated (BTPS) correction factors applied in the present study

Ambient temperature (°C)	Correction factor	Ambient temperature (°C)	Correction factor	Ambient temperature (°C)	Correction factor
15	1.128	23	1.085	31	1.039
16	1.123	24	1.08	32	1.032
17	1.118	25	1.075	33	1.026
18	1.113	26	1.069	34	1.02
19	1.108	27	1.063	35	1.014
20	1.102	28	1.057	36	1.007
21	1.096	29	1.051	37	1
22	1.091	30	1.045		

3.3.3.3.3 Quality control

Key performance characteristics of the measurement systems (linearity and dynamic response) were confirmed at the beginning and end of the study period, and remained stable. Details of linearity and dynamic response are given below.

3.3.4 Data analysis

The statistical concepts applied were guided by the texts of Streiner and Norman (2008), Domholdt (2005, pp. 243-274), Bland and Altman (1999) and Taylor (Taylor 1997).

To quantify instrument repeatability, *i.e.* the magnitude of measurement error attributable to the measurement instruments, the arithmetic mean of repeated measurements was calculated and

the error margin was described as mean ± ½ range, expressed both in the unit of measurement and as a percentage of the mean. This gives a conservative description, in which the extreme measured values are made transparent, as opposed to other options of describing error, such as mean ± standard deviation, mean ± standard error of the mean, or mean ± average deviation from the mean (Taylor 1997, pp. 13-44).

To quantify test-retest reliability, three methods of analysis were used. First, the discrepancy in measurements (first measurement – second measurement) was calculated for the examined parameters (FVC, FEV_1, PEF, PEmax, PImax, PECF of voluntary cough, and PECF of reflex cough). The variability in these parameters from the first to the second testing session was summarised using the method of Bland and Altman (1999), describing the mean difference and 95% limits of agreement.

Second, an intraclass correlation coefficient (ICC) was calculated. The ICC calculated was for comparison of absolute agreement (as opposed to consistency of agreement) and for comparison of individual measurements (as opposed to group average measurements). A mixed-effects model was used, treating the investigator as fixed, as the point of interest was to examine test-retest reliability for this particular investigator (as opposed to a population of potential investigators) (StataCorp 2013). These two analysis methods were used to complement each other. While ICC indicates the magnitude of reliability (greater reliability when ICC is closer to one), the method according to Bland and Altman conveys the actual magnitude of variability in the unit of measurement. A combination of both approaches allows for an evaluation of test-retest reliability against magnitudes of clinically relevant changes in test parameters (Rankin & Stokes 1998).

Third, a coefficient of variation was calculated for repeated measurements of PEmax, PImax, PECF of voluntary cough and PECF of reflex cough. This fractional statistic was calculated as |difference between two measurements|/mean of two measurements (Hankinson *et al*. 1998) and provides an indication of the magnitude of variation across the range of potentially relevant

values. Coefficients of variation were used to inform about the minimal detectable change in PEmax, PImax, PECF of voluntary cough and PECF of reflex cough.

Data analyses were conducted using Excel (Microsoft Office Excel 2007, Microsoft Corporation, Redmond, Washington) and Stata software (Stata v12.1, StataCorp, College Station, Texas).

3.4 Results

3.4.1 Forced spirometry

3.4.1.1 Instrument repeatability

The arithmetic mean ± ½ range (%) of ten consecutive measurements of three litre volumes delivered through the spirometer in the inspiratory direction of flow was: 3.04 ± 0.06 (2.0%) L in the flow range 0-54 L/min; 3.02 ± 0.04 (1.0%) L in the flow range 96-270 L/min; and 3.06 ± 0.02 (<1.0%) L in the flow range 420-720 L/min. In the expiratory direction of flow, it was 3.06 ± 0.06 (2.0%) L in the flow range 0-54 L/min; 2.97 ± 0.04 (1.0%) L in the flow range 96-270 L/min; and 3.04 ± 0.02 (<1.0%) L in the flow range 420-720 L/min.

3.4.1.2 Test-retest reliability in healthy subjects

The raw data for test-retest measurements in healthy subjects are presented in Table 9. The mean difference (95% limits of agreement) was 0.03 (-0.42, 0.48) L for FVC; 0.03 (-0.40, 0.46) L for FEV_1; and 24 (-70, 118) L/min for PEF. The ICC (95% confidence interval) was 0.98 (0.94, >0.99) for FVC; 0.96 (0.87, 0.99) for FEV_1; and 0.84 (0.50, 0.95) for PEF.

Table 9. Test-retest measurements for forced spirometry in healthy subjects

Subject	Forced vital capacity (L)		Forced expiratory volume in one second (L)		Peak expiratory flow (L/min)	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
1	4.77	4.77	3.84	3.94	652	608
2	4.35	4.49	3.72	3.73	560	572
3	3.03	3.52	2.69	3.19	410	472
4	2.90	2.62	2.45	2.16	494	404
5	5.94	6.14	4.03	4.01	449	475
6	3.18	3.00	2.44	2.43	398	364
7	4.67	4.51	3.57	3.43	549	486
8	2.58	2.37	1.99	2.03	362	344
9	4.82	4.80	3.85	3.75	442	409
10	6.09	5.88	4.29	3.97	629	538
11	4.10	4.01	3.76	3.70	473	481

3.4.1.3 Test-retest reliability in stroke patients

The raw data for test-retest measurements in stroke patients are presented in Table 10. The measurements are from six patients. All six carried out repeated tests approximately four weeks after stroke onset, and five performed repeated tests again approximately twelve weeks after stroke. The mean difference (95% limits of agreement) was -0.06 (-0.57, 0.45) L for FVC; -0.01 (-0.19, 0.17) L for FEV_1; and 6 (-49, 61) L/min for PEF. The ICC (95% confidence interval) was 0.96 (0.85, 0.99) for FVC; 0.99 (0.96, >0.99) for FEV_1; and 0.96 (0.85, 0.99) for PEF.

Table 10. Test-retest measurements for forced spirometry in stroke patients

Subject	Time post stroke	Forced vital capacity (L)		Forced expiratory volume in one second (L)		Peak expiratory flow (L/min)	
		Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
1	4 weeks	1.37	2.12	1.37	1.51	329	339
2	4 weeks	2.08	2.17	1.71	1.83	294	262
3	4 weeks	2.48	2.51	2.20	2.16	401	349
4	4 weeks	3.94	4.00	3.04	3.05	500	473
5	4 weeks	2.32	2.30	2.00	2.07	287	313
6	4 weeks	3.88	3.92	3.18	3.26	551	545
1	12 weeks	2.30	2.37	1.66	1.70	326	356
2	12 weeks	1.78	1.70	1.42	1.39	260	273
3	12 weeks	2.57	2.55	2.28	2.23	370	382
4	12 weeks	4.16	3.83	3.11	2.92	505	463
5	12 weeks	2.72	2.80	2.36	2.34	382	380

3.4.2 Maximal mouth pressure measurements

3.4.2.1 Instrument repeatability

The MicroRPM device was connected via a three-way airtight tubing system with a digital manometer (C9553 Pressure Meter, Comark, Norwich, England) and an inflation device (Encore 26 Inflator, Boston Scientific, Marlborough, Massachusetts, USA). Incremental positive and negative pressure was delivered to the system through the inflation device, and the MicroRPM measurement was compared against the digital manometer measurement. The arithmetic means ± ½ range (%) cmH_2O of ten repeated measurements at each pressure level are listed in Table 11. Of note, the MicroRPM device displays pressure (cmH_2O) in integers, accounting for the instances where error margins are zero.

Table 11. Instrument repeatability of the MicroRPM calculated from ten repeat measurements at each pressure level (cmH_2O). Of note, the MicroRPM device displays negative pressure (*i.e.* PImax measurements) in positive values.

'Known' pressure as per digital manometer	MicroRPM measurements (mean ± ½ range (%))
200	199.6 ± 1.5 (0.8%)
150	149.8 ± 1.0 (0.7%)
100	99.9 ± 1.0 (1.0%)
80	79.9 ± 0.5 (0.6%)
60	59.9 ± 0.5 (0.8%)
40	40.0 ± 0 (0%)
20	20.0 ± 0 (0%)
10	10.0 ± 0 (0%)
-10	10.0 ± 0 (0%)
-20	20.1 ± 0.5 (2.5%)
-40	40.1 ± 0.5 (1.2%)
-60	60.0 ± 0.5 (0.8%)
-80	80.2 ± 0.5 (0.6%)
-100	100.1 ± 1.0 (1.0%)
-150	150.3 ± 1.5 (1.0%)
-200	200.5 ± 1.5 (0.8%)

3.4.2.2 Test-retest reliability in healthy subjects

The raw data for test-retest measurements in healthy subjects are presented in Table 12. The mean difference (95% limits of agreement) was -2.2 (-19.6, 15.2) cmH_2O for PEmax and -5.5 (-23.7, 12.7) cmH_2O for PImax. The ICC (95% confidence interval) was 0.95 (0.84, 0.99) for PEmax and 0.95 (0.80, 0.99) for PImax.

Table 12. Test-retest measurements for maximal mouth pressures in healthy subjects

Subject	Maximal expiratory mouth pressure (cmH_2O)		Maximal inspiratory mouth pressure (cmH_2O)	
	Test 1	Test 2	Test 1	Test 2
1	129	138	105	112
2	109	108	118	118
3	65	77	63	68
4	104	107	57	48
5	151	148	84	84
6	65	69	44	52
7	133	130	80	75
8	120	141	95	103
9	145	144	151	162
10	125	121	100	128
11	106	95	98	105

3.4.2.3 Test-retest reliability in stroke patients

The raw data for test-retest measurements in stroke patients are presented in Table 13. The measurements are from six patients. All six carried out repeated tests approximately four weeks after stroke onset, and five performed repeated tests again approximately twelve weeks after stroke. The mean difference (95% limits of agreement) was -2.2 (-14.9, 10.5) cmH_2O for PEmax and 1.1 (-6.9, 9.1) cmH_2O for PImax. The ICC (95% confidence interval) was 0.94 (0.79, 0.98) for PEmax and 0.96 (0.88, 0.99) for PImax.

Table 13. Test-retest measurements for maximal mouth pressures in stroke patients

Subject	Time post stroke	Maximal expiratory mouth pressure (cmH_2O)		Maximal inspiratory mouth pressure (cmH_2O)	
		Test 1	Test 2	Test 1	Test 2
1	4 weeks	70	80	43	39
2	4 weeks	53	63	36	37
3	4 weeks	97	89	43	42
4	4 weeks	106	109	75	66
5	4 weeks	69	76	51	55
6	4 weeks	94	88	74	78
1	12 weeks	106	107	47	45
2	12 weeks	64	67	30	32
3	12 weeks	89	94	47	48
4	12 weeks	115	108	75	69
5	12 weeks	72	78	58	56

3.4.3 Cough flow measurements

3.4.3.1 Linearity of the measurement system

Linearity of the on-site and the off-site cough measurement systems was assessed in both directions of flow through the pneumotachograph. A mechanical flow generating device (Numatic NVDQ572, 1700W, Numatic International, Chard, England) and a rotameter (Rotameter 2000, TM-47X FM-A, 80-760 L/min flow range, Process Instruments Ltd, Croydon, England) were used to deliver steady airflow in increments of 100 L/min. The corresponding voltage recorded with the on-site measurement system at each flow level is presented in Figure 6. The system showed good linearity with an r^2 (square of the Pearson correlation coefficient) of 0.999148. For the off-site measurement system, r^2 was 0.999376. Good linearity justified the use of two-point calibration.

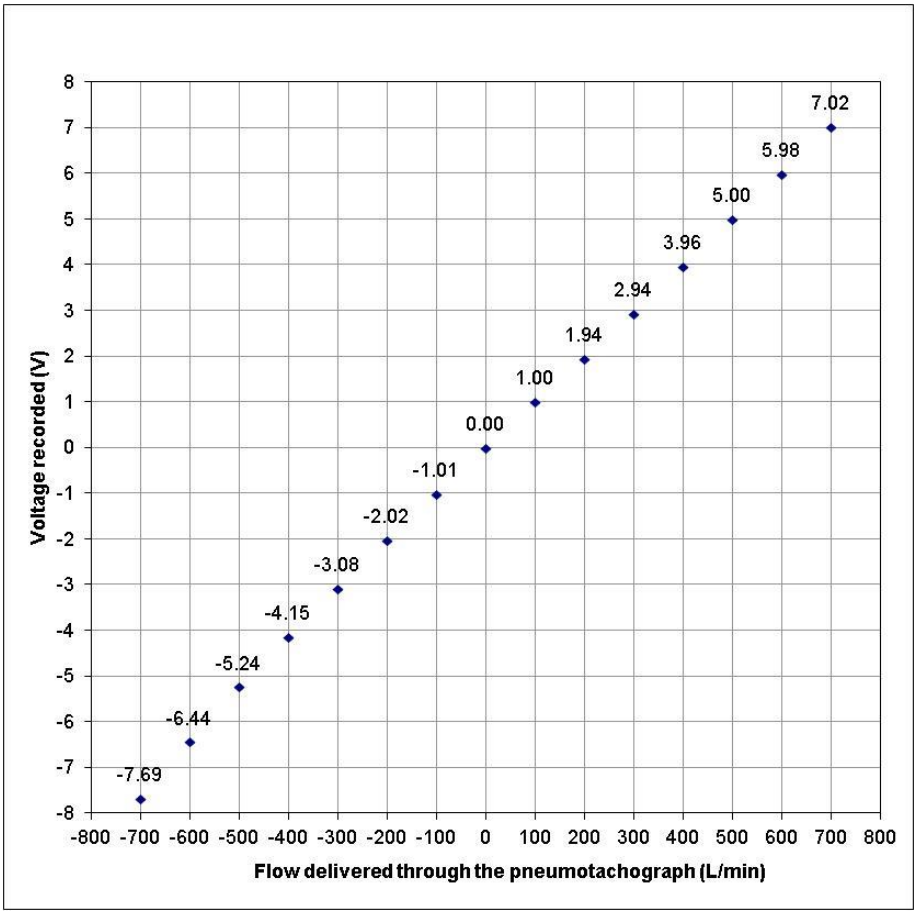


Figure 6. Linearity of the cough flow measurement system, $r^2 = 0.999148$

3.4.3.2 Dynamic response of the measurement system

The on-site and the off-site cough measurement systems' responses to rapid change in air flow were assessed. During the expulsive phase of cough, expiratory flow peaks within 0.025 to 0.050 seconds. The measurement system needs to be sufficiently sensitive to capture this rapid increase in signal and not to 'miss' the transient flow peak.

Dynamic response was evaluated through rapid cessation of flow (Miller *et al*. 2003, 2002) using the 'pop-test' method (Ward 2012, pp. 90-91). An inflated toy balloon was connected to the pneumotachograph via a valve and corrugated tubing (ID 20.0 mm, length 40.0 cm). Steady

flow was generated from the balloon and rapidly discontinued by bursting the balloon with a needle.

For the on-site measurement system, voltage dropped to zero in 0.0101 seconds from when flow was discontinued. For the off-site measurement system, the trace reached zero 0.024 seconds after cessation of flow. For both systems, the response time to sudden cessation of flow was adequate to capture the rapid rise in expiratory flow during cough.

3.4.3.3 Instrument repeatability

3.4.3.3.1 Repeatability of flow measurements

Steady flow was delivered through the pneumotachograph in 100 L/min increments from -700 L/min to 700 L/min, using a mechanical flow generating device (Numatic NVDQ572, 1700W, Numatic International, Chard, England) and a rotameter (Rotameter 2000, TM-47X FM-A, 80-760 L/min flow range, Process Instruments Ltd, Croydon, England). The arithmetic means ± ½ range (%) L/min of ten repeated measurements at each flow level are listed in Table 14. Means ± SD for the off-site measurement system are shown in Figure 7. Systems were calibrated anew before each measurement, so that the variability observed here includes error related to the flow calibration procedure.

Table 14. Repeatability of steady flow measurements calculated from ten repeat measurements at each flow level (L/min).

'Known' flow as per rotameter	Measurements (mean ± ½ range (%))	
	On-site measurement system	Off-site measurement system
700	723 ± 28 (3.9%)	737 ± 13 (1.8%)
600	611 ± 20 (3.3%)	621 ± 7 (1.1%)
500	500 ± 13 (2.5%)	507 ± 9 (1.8%)
400	396 ± 8 (2.0%)	403 ± 8 (2.0%)
300	290 ± 5 (1.7%)	297 ± 8 (2.7%)
200	191 ± 3 (1.6%)	196 ± 6 (3.1%)
100	96 ± 2 (2.6%)	99 ± 6 (6.0%)
-100	-95 ± 3 (2.9%)	-100 ± 8 (8.0%)
-200	-188 ± 8 (4.4%)	-192 ± 10 (5.2%)
-300	-283 ± 15 (5.3%)	-288 ± 15 (5.2%)
-400	-384 ± 26 (6.8%)	-390 ± 26 (6.7%)
-500	-481 ± 36 (7.5%)	-490 ± 32 (6.5%)
-600	-585 ± 49 (8.4%)	-606 ± 41 (6.8%)
-700	-693 ± 65 (9.4%)	-711 ± 54 (7.6%)

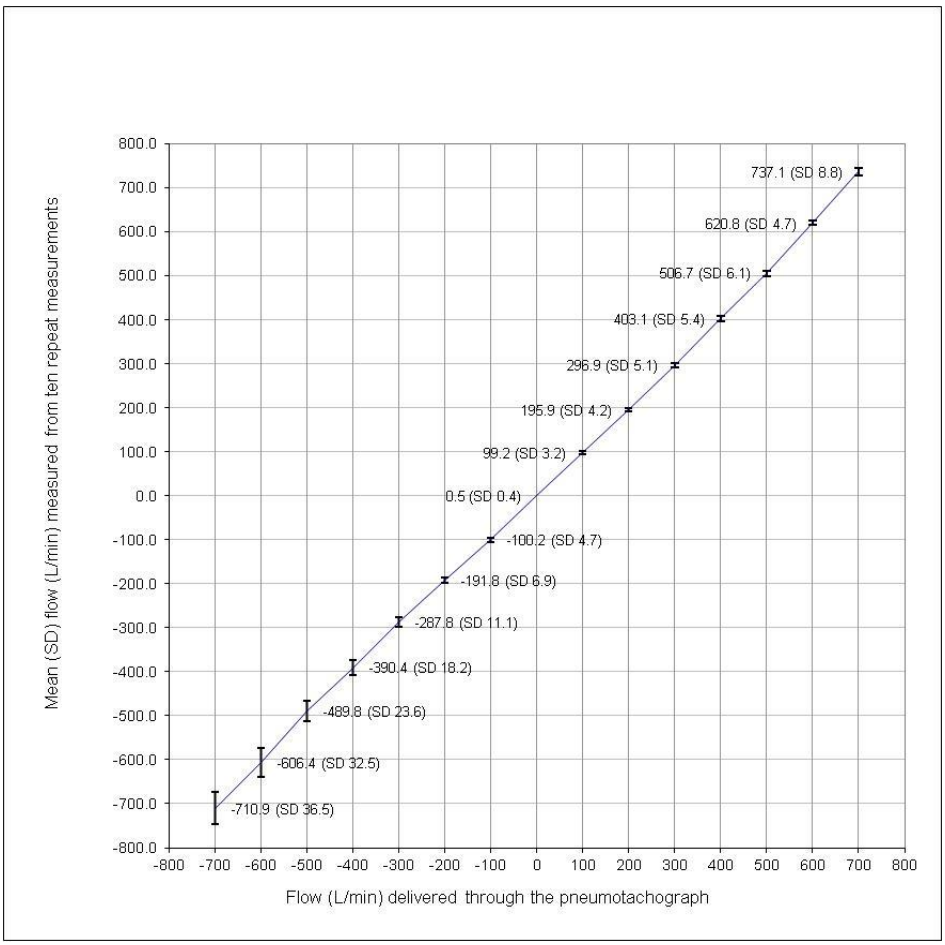


Figure 7. Calibration curve. Error bars represent standard deviation.

3.4.3.3.2 Repeatability of volume measurements

Volumes of 0.1 L, 0.5 L, 1.5 L and 3.0 L were delivered through the pneumotachograph in inspiratory and expiratory direction of flow at randomly altered flow levels using a three-litre calibration syringe. The arithmetic means ± ½ range (%) of ten consecutive measurements for each volume and in the respective directions are listed in Table 15.

Table 15. Repeatability of volume measurements calculated from ten repeat measurements for each volume (L)

	'Known' volume delivered (L)	Flow range (L/min)	Measurements in inspiratory direction (mean (L) ± ½ range (%))	Measurements in expiratory direction (mean (L) ± ½ range (%))
On-site measurement system	0.1	19-180	0.1 ± <0.01 (6.0%)	0.1 ± 0.01 (10.0%)
	0.5	32-305	0.5 ± <0.01 (1.0%)	0.5 ± 0.01 (2.0%)
	1.5	40-680	1.5 ± 0.01 (0.7%)	1.4 ± 0.03 (2.1%)
	3.0	59-1025	3.0 ± 0.03 (1.0%)	2.9 ± 0.06 (2.1%)
Off-site measurement system	0.1	20-146	0.1 ± <0.01 (3.9%)	0.1 ± <0.01 (6.8%)
	0.5	35-363	0.5 ± 0.01 (2.0%)	0.5 ± 0.02 (3.2%)
	1.5	51-728	1.5 ± <0.01 (0.4%)	1.5 ± 0.04(2.4%)
	3.0	64-1086	3.0 ± <0.01 (0.2%)	3.0 ± 0.08 (2.8%)

3.4.3.3.3 Peak expiratory flow measurements with and without the nebuliser circuit

The pneumotachograph system had higher airflow resistance when reflex cough flow was measured, due to the two-way non-rebreathing valve and filter connected downstream to the pneumotachograph. Given consistent driving pressure, higher airflow resistance will result in lower flow, and vice versa. Therefore, the influence of the nebuliser circuit on absolute values of peak flow measurements was experimentally determined by measuring the peak flow of short duration flow bursts. Flow bursts were generated with a 50 L pressure vessel (Medical Engineering Department, Royal Brompton Hospital, London, England) connected to a balloon occlusion valve (Medical Engineering Department, Royal Brompton Hospital, London, England). The vessel was pressurised with compressed air to a predetermined pressure, monitored with a digital manometer (C9553 Pressure Meter, Comark, Norwich, England), at which point the occlusion valve was opened and a burst of short duration airflow released. The consistency of peak flow for these flow bursts was confirmed with five consecutive measurements with the pneumotachograph system. Incremental vessel pressures from 25 to 800 mmH_2O were used.

At each level of pressure, peak flow was measured with the pneumotachograph with and without attached nebuliser circuit. Differences in measurements are shown in Table 16 and Figure 8. The measurements with nebuliser circuit attached are of a magnitude of between 65% and 69% of the corresponding measurements without nebuliser circuit, so that the added resistance of the nebuliser circuit caused a systematic reduction of the absolute peak flow measurement by between 35% and 31%. This needs to be considered when interpreting absolute peak cough flow values for voluntary cough (measured with pneumotachograph without attached nebuliser circuit) and reflex cough (measured with pneumotachograph with attached nebuliser circuit).

Table 16. Peak flow measurements (L/min) of short duration flow bursts, comparing the pneumotachograph with and without attached nebuliser circuit

Driving pressure for flow burst generation (mmH_2O)	Peak flow measurements (L/min)	
	Pneumotachograph	Pneumotachograph and attached nebuliser circuit (% of measurement without attached nebuliser circuit)
25	72	47 (65%)
50	131	90 (69%)
100	243	163 (67%)
150	336	220 (65%)
200	427	281 (66%)
250	490	321 (66%)
300	553	361 (65%)
350	607	397 (65%)
400	662	432 (65%)
450	708	468 (66%)
500	746	503 (67%)
550	793	538 (68%)
600	840	572 (68%)
650	884	608 (69%)
700	934	638 (68%)
750	971	670 (69%)
800	1010	697 (69%)

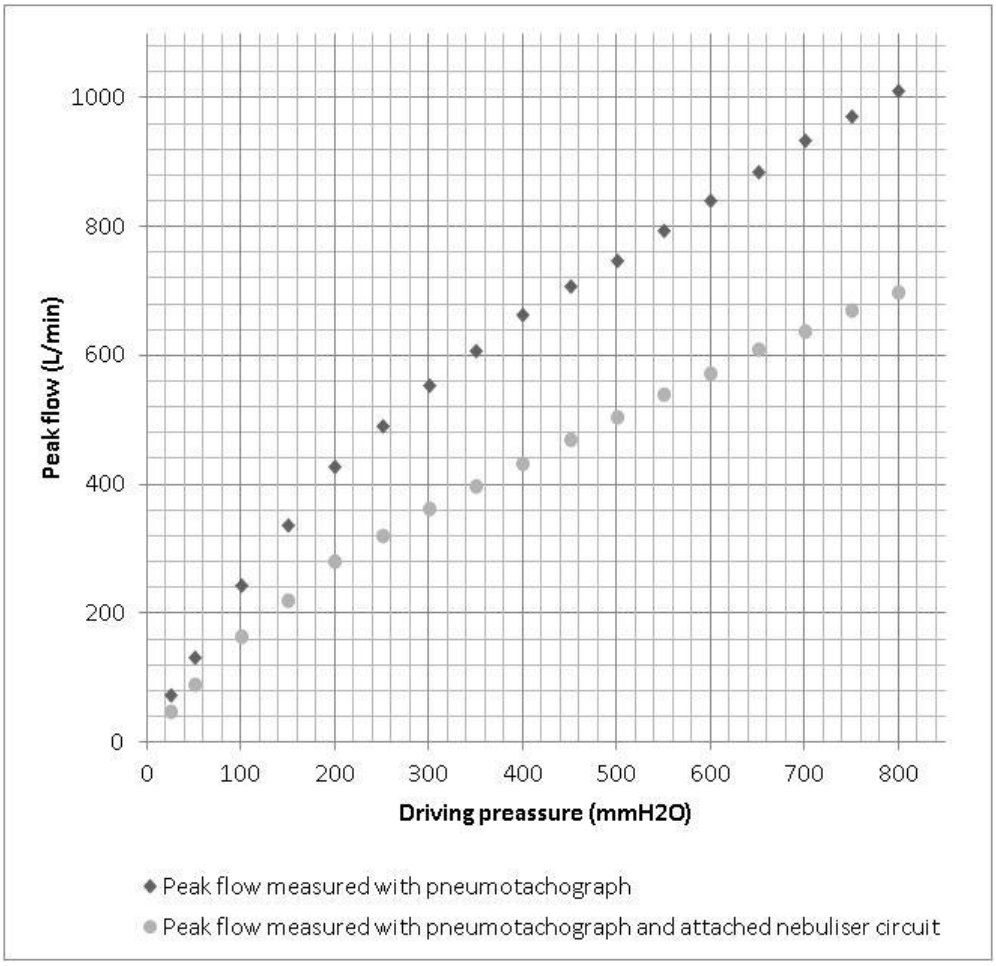


Figure 8. Peak flow measurements (L/min) of short duration flow bursts, comparing the pneumotachograph with and without attached nebuliser circuit.

3.4.3.4 Test-retest reliability in healthy subjects

Test-retest reliability was examined for PECF of voluntary and reflex cough, as these were the primary parameters of interest for the subsequent clinical studies. The raw data for test-retest measurements in healthy subjects are presented in Table 17. The mean difference (95% limits of agreement) was -15 (-199, 169) L/min for PECF of voluntary cough and 4 (-153, 161) L/min for PECF of reflex cough. The ICC (95% confidence interval) was 0.94 (0.79, 0.98) for PECF of voluntary cough and 0.64 (0.02, 0.90) for PECF of reflex cough.

Table 17. Test-retest measurements of peak expiratory cough flow (PECF) in healthy subjects

Subject	PECF of maximal voluntary cough (L/min)		PECF of capsaicin-induced reflex cough (L/min)	
	Test 1	Test 2	Test 1	Test 2
1	1221	1129	599	425
2	1082	1183	350	381
3	521	578	252	390
4	800	808	307	267
5	896	914	-	-
6	625	600	269	309
7	1211	1094	396	407
8	540	573	256	200
9	905	1030	373	405
10	833	994	276	250
11	561	458	318	324

Note: no reflex cough was elicited in subject 5 at the highest available concentration of capsaicin

3.4.3.5 Test-retest reliability in stroke patients

The raw data for test-retest measurements in stroke patients are presented in Table 18. The measurements are from six patients. All six carried out the tests approximately four weeks after stroke onset, and five performed the tests again approximately twelve weeks after stroke. The mean difference (95% limits of agreement) was 44 (-42, 130) L/min for PECF of voluntary cough and 25 (-181, 231) L/min for PECF of reflex cough. The ICC (95% confidence interval) was 0.98 (0.86, 0.99) for PECF of voluntary cough and 0.68 (0.18, 0.90) for PECF of reflex cough.

Table 18. Test-retest measurements of peak expiratory cough flow (PECF) in stroke patients

Subject	Time post stroke	PECF of maximal voluntary cough (L/min)		PECF of capsaicin-induced reflex cough (L/min)	
		Test 1	Test 2	Test 1	Test 2
1	4 weeks	457	467	408	277
2	4 weeks	332	307	237	182
3	4 weeks	659	587	362	379
4	4 weeks	934	958	488	533
5	4 weeks	851	790	487	379
6	4 weeks	942	839	305	463
1	12 weeks	525	512	281	307
2	12 weeks	313	262	219	112
3	12 weeks	666	682	388	377
4	12 weeks	966	959	666	470
5	12 weeks	853	764	271	360

3.4.3.6 Comparability of the on-site and off-site measurement systems

Linearity, dynamic response and instrument repeatability were examined for both the on-site and off-site measurement systems. The two systems had equivalent linearity. The on-site system was approximately twice as responsive to rapid change in signal as the off-site system; however, both systems met the dynamic response requirements for cough testing. With respect to the repeatability of flow and volume measurements the two systems differed in some aspects within an overall adequate performance level.

In the evaluation of test-retest reliability in healthy subjects and stroke patients, both the on-site and off-site measurement systems were used, depending on the location of the subject. These data therefore reflect the actual circumstances of measurement in the subsequent clinical studies.

3.4.4 Minimal detectable difference for cough flow and maximal mouth pressure measurements

To inform the interpretation of change over time, coefficients of variation were calculated using the test-retest data from healthy subjects and stroke patients for PEmax, PImax, PECF of voluntary cough and PECF of reflex cough. These four parameters were selected, as they were primary parameters of interest in subsequent clinical studies. The coefficient of variation indicates the difference in two consecutive measurements that can be expected in the absence of 'true' change. This statistic allows test-retest variability to be described in relation to the magnitude of measurements on the scale of potential values (as opposed to Bland-Altman statistics, which summarise the mean and spread of differences for the entire range of observed values). Coefficients of variation are given in Table 19.

Table 19. Coefficients of variation for maximal mouth pressure and cough flow measurements

	Mean	Min	Max
Healthy subjects			
PECF of voluntary cough	0.09	0.01	0.20
PECF of reflex cough	0.16	0.02	0.43
PEmax	0.06	<0.01	0.16
PImax	0.09	<0.01	0.24
Stroke patients			
PECF of voluntary cough	0.07	0.01	0.18
PECF of reflex cough	0.26	0.03	0.65
PEmax	0.08	0.01	0.17
PImax	0.06	0.02	0.13

The interpretation of these coefficients is that, *e.g.* for healthy subjects the group mean test-retest variability for PECF of voluntary cough was 0.09. An observed change over time that exceeds this error margin of ± 0.09 or 9% can therefore be interpreted as 'true' change, rather than variability attributed to test-retest variability in the absence of change. As the most conservative estimate of test-retest variability, the highest observed coefficient of variability can be taken to describe the error margin, *e.g.* 0.20 or 20% for PECF of voluntary cough in healthy subjects.

3.5 Discussion

The series of method validation experiments described in this chapter addresses the issue of measurement variability for three respiratory assessment methods: forced spirometry, measurement of maximal mouth pressures and cough flow measurement. Forced spirometry and maximal mouth pressure measurements are commonly used in clinical practice and research, and detailed guidelines on the standardisation of these test procedures are available (Miller *et* al. 2005, American Thoracic Society (ATS) & European Respiratory Society (ERS) 2002). In contrast, the measurement of cough flow is less commonly applied and there are no guidelines for the standardisation of cough flow testing. Measurement error can be reduced by standardising test procedures. Table 20 lists potential sources of measurement variability relating to the cough flow measurement systems and test procedures applied in the present studies. The investigator was conscious of these issues throughout the research process and took care to minimise measurement error arising from these sources.

Table 20. Sources of measurement error when measuring cough flow in human subjects

Instrument	Comments
Vertical position of the rotameter when calibrating the system	This consideration was particularly relevant when setting up the measurement system in participants' homes, where a convenient stable horizontal surface was not always readily available.
Geometry of connecting components	The size and shape of connecting components influence the performance characteristics of the measurement system (Jackson & Vinegar 1979, Finucane *et al*. 1972) and were therefore not altered for the duration of the study.
Steady reference flow for flow calibration	Negative flow (*i.e.* vacuum) applied to the rotameter and pneumotachograph shows less oscillation than positive flow, and therefore provides a steadier reference point for calibration.
Direction of flow through the pneumotachograph when delivering reference flow for calibration and when testing	Linearity of the measurement system was somewhat better in expiratory direction of flow through the pneumotachograph. Since expiratory cough flow was more relevant as an outcome for the studies than inspiratory cough flow, the reference flow when calibrating was applied to the pneumotachograph in the same direction as the subject's expiratory cough flow. This maximised precision of expiratory flow measurements.
Fit of the face mask	Inadequate fit of the face mask or movement of the mask during testing could lead to air leak and measurement variability. Care was taken during testing to monitor the fit of the mask throughout testing.
Intra-subject	**Comments**
Location	Room temperature, humidity and atmospheric pressure affect the measurement of air flow. From these, room temperature is the most relevant factor and was corrected for using the appropriate correction factor.
Body position	Body position (*i.e.* supine versus sitting position) influences lung volumes (Dean 2002) and therefore may also influence cough flow rates. Testing was always conducted with the test subject in a sitting position, either on a chair, armchair or positioned upright in the hospital bed.

Table 20. continued

Changes in mood, motivation, effort, alertness, fatigue, pain	Daily constitution could influence performance of voluntary cough tests. In a population of acute stroke patients, alertness, fatigue, mood and pain are particularly relevant factors, which can fluctuate considerably in the acute phase of the condition. The investigator took note of subjects' level of alertness and any indication of fatigue, mood, or pain influencing measurements.
Biological/physiological variation	Variability may be due to biological and physiological variation beyond the control or perception of the subject and investigator.
Intra-rater	Comments
Parallax	Differing line of sight when reading the calibration reference flow off the rotameter bobbin could introduce variability. This consideration was particularly relevant when calibrating at a subject's home, where the height of surfaces could require the operator to crouch, stoop or lean sideways in order to align line of sight with the rotameter scale.
Consistency of verbal instructions to the subject	The quality of the operator's verbal instructions could influence the consistency of measurements. More assertive and enthusiastic instructions usually result in a more motivated volitional effort by the test subject. Including the instruction to take in a deep breath prior to coughing likely results in higher expiratory cough flow, as expiratory cough flow in part depends on inspired volume. Care was taken to give consistent instructions to subjects throughout the study period.
Extracting data from software	Variability in marking points of measurement by hand was reduced by using automated functions when possible (*e.g.* maximum and minimum value of a selected portion). However, some parameters had to be extracted from cough flow traces by positioning markers by hand. Care was taken to apply consistent procedures when extracting data from cough flow traces, *e.g.* using the same level of screen magnification when positioning markers.
Inter-rater	Comments
Inter-rater variability was not relevant to the present study, as all measurements were performed by one investigator.	In principle, all points relating to intra-rater variability also apply to inter-rater variability, and are likely to affect variability to an even greater extent.

The measurement error attributable to the performance of measurement instruments was evaluated through instrument repeatability bench tests. Instrument repeatability was excellent for the spirometer (volume accuracy) and the MicroRPM device (pressure accuracy), with error margins below 2% across the range of measurements. The flow measurement systems were shown to have adequate linearity and dynamic response. Flow accuracy of the cough flow measurement systems was good in the expiratory direction of flow from 200 L/min upwards, with conservative error margins estimates of below 4%. In the 100 L/min flow range in the expiratory direction error margins were below 6%, and in the inspiratory direction of flow error margins were 5% to 9% across the range. Volume accuracy of the cough flow measurement system was good for volumes from 0.5 to 3.0 L, with error margins of up to 2% for inspiratory volumes and up to 3% for expiratory volumes. For small volumes of 0.1 L, error margins were up to 6% and 10% for inspiratory and expiratory volumes, respectively. The salient point from these data is that for the parameters that were most relevant in subsequent clinical studies (peak expiratory flow of cough and maximal mouth pressures) the instruments showed a good level of precision. The MicroRPM was able to measure maximal mouth pressure accurately to within ± 2%, and the cough flow measurement system was able to measure expiratory flow accurately to within ± 4%.

As to be expected, test-retest reliability data from human subjects showed a greater level of variability than instrument repeatability. Variability as quantified through ICCs, Bland-Altman statistics and coefficients of variation was of approximately equivalent magnitude in healthy subjects and in stroke patients, which demonstrates that these respiratory assessments can be performed as reliably in the clinical population of interest for the present studies as in healthy subjects.

ICCs were high throughout with values >0.90 for most parameters, except for PEF in healthy subjects, where the ICC was 0.84, and PECF of reflex cough in healthy and stroke subjects, where ICCs were 0.64 and 0.68, respectively. Bland-Altman statistics and coefficients of variation informed about the magnitude of variability (in the unit of measurement) in the absence of ‘true’ change. For example, for the test-retest measurements of PECF of voluntary cough in

stroke patients, the mean difference (first measurement – second measurement) was 44 L/min, indicating that there was a trend towards lower cough flow measurements at the second time of testing, which may be interpreted as an element of fatigue. In 95% of observations, the difference was between -42 and 130 L/min (95% limits of agreement). This variability may seem high, considering that measurements in the flow range of 200, 300 and 400 L/min can be expected in stroke patients with impaired cough function. However, 95% limits of agreement summarise the discrepancy between measurements across the entire scale of possible measurements. The coefficient of variation statistic shows that measurement variability was smaller in absolute terms at the lower end of the scale and higher in absolute terms at the higher end of the scale. For example, for PECF of voluntary cough in stroke patients, the mean coefficient of variability was 0.07, which corresponds to error margins of ± 14 L/min, ± 28 L/min, ± 42 L/min and ± 56 L/min at 200, 400, 600 and 800 L/min flow, respectively. Using the highest observed coefficient of variability for PECF of voluntary cough in the stroke group (0.18) for the most conservative estimate of test-retest variability, error margins were ± 36 L/min, ± 72 L/min, ± 108 L/min and ± 144 L/min at 200, 400, 600 and 800 L/min flow, respectively. The interpretation of these statistics is that, *e.g.* for measurements in the flow range of 200 L/min an observed difference of >36 L/min can be attributed to 'true' change beyond the level of expected test-retest variability, whereas in the flow range of 800 L/min the difference observed needs to exceed 144 L/min to be interpreted as 'true' change, etc. These data correspond with the variability for PECF measurements observed by Singh *et al*. (1994), who reported coefficients of variability ranging from 0.09 to 0.23 (derived from 15 PECF measurements made over a four-week period in twelve healthy volunteers).

For maximal expiratory and inspiratory mouth pressure measurements in stroke patients, the mean coefficients of variation were 0.08 and 0.06, respectively. The highest coefficients of variation were 0.17 for PEmax and 0.13 for PImax. As the most conservative estimates therefore, expected error margins for PEmax in stroke patients are ± 3 cmH_2O, ± 7 cmH_2O, ± 10 cmH_2O and ± 14 cmH_2O at 20, 40, 60 and 80 cmH_2O, respectively; and expected error margins for PImax are ± 3 cmH_2O, ± 5 cmH_2O, ± 8 cmH_2O and ± 10 cmH_2O at 20, 40, 60 and 80 cmH_2O, respectively. Of note, these figures indicate the minimal detectable difference based on

measurement variability, but not the minimally important difference (*i.e.* the smallest change in the respective parameters that is considered clinically relevant).

Measurements of PECF of reflex cough showed the highest degree of test-retest variability, with ICCs of 0.068 and 0.064 in healthy volunteers and stroke patients, respectively. 95% limits of agreement in the stroke group were -181 L/min to 231 L/min. The mean coefficient of variation in the stroke group was 0.26, and the highest coefficient of variation in the stroke group was 0.65. This high test-retest variability for reflex cough flow measurements can be explained by the nature of the test. Factors contributing to limited reproducibility of inhalation cough challenge tests have been described (Morice *et al*. 2007, pp. 1258-1261). Variability in subjects' readiness to allow or suppress the urge to cough may contribute to measurement variability. Some subjects may respond with hesitation when asked to inhale the irritant. This contributes to variability in respiratory flow and volumes, which in turn influence the actual dose of the irritant delivered to the target receptor sites in the laryngeal and tracheal region. In addition, receptor sensitivity to capsaicin can adapt over the short term, which may lead to variability in the force of elicited reflex responses. The moment at which a reflex cough is triggered during the breathing cycle is unpredictable. This means that coughs can be elicited at different points during inspiration and expiration, when the volume of air within the lungs is greater or lesser. This influences the peak cough flow achieved, since expiratory cough flow is dependent on both driving pressure (*i.e.* force of expiratory muscle contraction) and pre-cough inspiratory volume.

3.6 Conclusion

In conclusion, the series of validation experiments described in this chapter addressed issues of measurement variability for three respiratory assessment procedures: forced spirometry, measurement of maximal mouth pressures and cough flow measurement. Instrument repeatability and test-retest reliability in healthy subjects and in acute stroke patients were examined. The most relevant measurement parameters for subsequent clinical studies were PECF of voluntary cough, PECF of capsaicin-induced reflex cough, PEmax and PImax.

Instrument repeatability was good, with conservative error estimates of <4% for expiratory flow measurements using the cough flow measurement systems; and <2% for pressure measurements using the MicroRPM device for maximal mouth pressure measurement. The cough flow measurement systems showed adequate linearity and dynamic response.

Test-retest reliability was of approximately equivalent magnitude in healthy subjects and in stroke patients, demonstrating that these respiratory assessments can be performed as reliably in the clinical population of interest for the present studies as in healthy subjects. Test-retest reliability was high for PECF of voluntary cough, PEmax and PImax (ICCs >0.90), and moderate for PECF of reflex cough (ICCs 0.60 to 0.70).

The magnitude of test-retest variability in the unit of measurement was described using Bland-Altman statistics (mean difference, 95% limits of agreement) and the coefficient of variation. Accordingly, the minimal detectable difference, taking into account all possible sources of measurement variability (instrument, intra-rater and intra-subject) and using the mean coefficient of variation observed in stroke patients, is: >7% change in PECF of voluntary cough; >26% change in PECF of reflex cough; >8% change in PEmax; and >6% change in PImax.

These data informed the design, analysis and interpretation of the subsequent clinical studies.

Chapter 4 Accuracy of portable flow measurement devices for the assessment of peak cough flow

4.1 Introduction

Cough flow testing is useful as an outcome measure in research and a monitoring or diagnostic tool in clinical practice. Peak expiratory cough flow (PECF) is commonly used as an indicator of the strength or effectiveness of cough, particularly in clinical populations with neuromuscular impairment (Jones *et al.* 2012). Cough can be accurately quantified using laboratory pneumotachograph based systems as described by Singh *et al.* (1994), but these can often consist of several components, can be expensive, not easily transportable, and require significant knowledge by the user for correct operation. Practical devices, which can conveniently be applied in clinical settings, patients' homes or other community locations, may be of use to clinicians and researchers. In several clinical studies, standard peak flow meters and hand-held spirometers have been used to measure PECF (Table 21). These devices are designed to measure peak flow during a forced expiratory manoeuvre, and their accuracy in measuring peak flow during cough is uncertain.

Table 21. Portable peak flow meters and spirometers used for the measurement of peak cough flow in clinical research.

Device	Study	Study population
Peak flow meters		
AsmaPLAN (Vitalograph, Ennis, Ireland)	LoMauro *et al.* 2014	Duchenne muscular dystrophy
	Sancho *et al.* 2004	Neuromuscular disease, healthy subjects
Assess (Philips Respironics, Pittsburgh, Pennsylvania) [a]	Cleary *et al.* 2013	Amyotrophic lateral sclerosis
	Bach *et al.* 2006	Restrictive pulmonary syndrome due to neuromuscular disease
	Kang *et al.* 2006b	Cervical spinal cord injury
	Bach *et al.* 1997	Duchenne muscular dystrophy
	Bach & Saporito 1996	Spinal cord injury, progressive neuromuscular disease
	Bach 1995	Amyotrophic lateral sclerosis
Astech (Astech, New York, New York)	Daftary *et al.* 2007	Duchenne muscular dystrophy
MicroPeak (Micro Medical Ltd, Rochester, England)	Lee *et al.* 2013	Traumatic brain injury, healthy subjects
Mini-Wright (Clement Clarke International, Harlow, England)	Silverman *et al*. 2014	Healthy subjects, Parkinson's disease
	Cardoso *et al.* 2012	Healthy subjects
	Freitas *et al*. 2010	Healthy elderly subjects
	Brito *et al*. 2009	Duchenne muscular dystrophy

Table 21. continued

Mini-Wright DIGITAL (www.miniwrightpeakflowmeter.com)	Silverman *et al*. 2014	Healthy subjects, Parkinson's disease
Wright (Wright & McKerrow 1959)	Gauld & Boynton, 2005	Duchenne muscular dystrophy
	Leiner *et al*. 1966	Obstructive and/or restrictive pulmonary disease, healthy subjects
Personal Best (Philips Respironics, Pittsburgh, Pennsylvania)	Suarez *et al*. 2002	Duchenne muscular dystrophy, amyotrophic lateral sclerosis
Pocketpeak (Ferraris Medical Ltd, Enfield, England)	Dohna-Schwake *et al*. 2006	Muscular dystrophies
Spirometers		
Autospiro AS-505 (Minato Medical Science, Osaka, Japan)	Kimura *et al*. 2013	Stroke
Micro-S 2000 (C. Schatzman, Madrid, Spain)	Sancho *et al*. 2007	Amyotrophic lateral sclerosis
Spirobank (Medical International Research, Rome, Italy)	Fiore *et al*. 2008	Cardiac surgery

[a] Formerly manufactured as Access Model 710 peak flow meter (Health Scan Products Inc, Cedar Grove, New Jersey, USA)

4.2 Aims and objectives

A series of experiments was conducted to examine the accuracy of two commonly used peak flow meters and one hand-held spirometer when measuring PECF. The purpose was to investigate whether the pneumotachograph-based measurement system used in subsequent clinical studies, which provided physiologically detailed measurements but consisted of an elaborate and expensive equipment setup, could potentially be substituted with convenient and less expensive hand-held clinical flow measurement devices.

The specific objectives were to:

- Describe the accuracy of devices when measuring PECF
- Explore potential sources of the observed inaccuracy.

4.3 Methods

4.3.1 Study design

Three hand-held devices were examined: the Assess peak flow meter (range 60-880 L/min, accuracy ±10% or 20 L/min according to manufacturer, Philips Respironics, Pittsburgh, Pennsylvania); the Mini-Wright Standard peak flow meter (European Union (EU) Scale, range 60-800 L/min, accuracy ±10% or 10 L/min according to manufacturer, Clement Clarke International, Harlow, England); and the SpiroUSB turbine spirometer, (range 12-900 L/min, accuracy ±3% according to manufacturer, CareFusion, San Diego, California). These three test devices were selected as they are produced by leading manufacturers and frequently used in clinical practice.

Three experiments were conducted to assess the accuracy of PECF measurements, and each of these has advantages and limitations. The first approach was to compare PECF measurements from healthy subjects' maximal volitional coughs, which were recorded with each

of the test devices in turn. The limitation of this approach is that findings are biased by error due to intra-subject variability of repeated maximal volitional cough efforts.

The second approach was to connect each test device in series with a calibrated pneumotachograph. Healthy subjects produced cough efforts at different levels of intensity through the setup. For each cough manoeuvre, PECF readings from the pneumotachograph and the test device were compared. In this approach, the pneumotachograph measurement was taken as the gold standard method of measurement. The advantage of this approach is that PECF readings from coughs of various intensity can conveniently be compared between the pneumotachograph and second device. The limitation of this approach is that the in-series connection of pneumotachograph and second device may introduce error due to alteration of airflow characteristics and instrument performance.

The third approach was to mechanically generate short duration flow bursts, the flow-time traces of which resembled those of human coughs. These simulated ‘coughs’ could be reproduced with consistent peak flow and discharged repeatedly into each of the test devices in turn. The advantage of this approach is that the peak flow of these simulated ‘coughs’ can be conveniently varied across the range of clinically relevant peak flow values. The limitation of this approach is that these mechanically generated ‘coughs’ may not represent the entire range of relevant physical characteristics of human cough, although key parameters (peak flow, rise time, volume) were within the range observed in human coughs.

4.3.2 Participants

Healthy adults with no medical history of respiratory disease or conditions affecting the anatomy and function of the upper airway who were comfortable coughing repeatedly over a short period of time were recruited. The study had ethical approval from the Psychiatry, Nursing and Midwifery Research Ethics Committee at King’s College London, UK (study reference PNM/12/13-143). All participants gave written informed consent.

4.3.3 Data collection

For the first experiment, a bacterial filter (Spiroguard Standard, Air Safety Medical, Morecambe, England) was connected to the devices under test. The calibration of the SpiroUSB spirometer was verified with a three-litre calibration syringe at the beginning of each testing session as per manufacturer's recommendation Subjects were seated comfortably and gave maximal volitional coughs (investigators instruction: 'Take a deep breath in and give a strong cough') through the open port of the bacterial filter, while maintaining a good lip seal around the port. For each of the three test devices, participants made five consecutive maximal cough efforts, and the highest PECF measurement was taken for analysis. Test devices were rotated in random order.

For the second experiment, the bacterial filter, a pneumotachograph and one test device were connected in series. The pneumotachograph system consisted of a Fleisch-type pneumotachograph (ID 4.4 cm, length 6.0 cm, PK Morgan Ltd, Rainham, England). Differential pressure was measured using a Validyne differential pressure transducer (MP45, range ± 2 cmH2O, Validyne Engineering, Northridge, CA) and the signal amplified (CD15, Validyne Engineering, Northridge, CA) and acquired on a laptop running LabChart software (LabChart Pro, version 7.2.2, ADInstruments Ltd, Oxford, England) with analog-to-digital sampling of 2 kHz (PowerLab/16SP, ADInstruments Ltd, Oxford, England). The pneumotachograph system was linear in the flow range from zero to 700 L/min (r^2 = 0.999845). A two-point calibration was performed at the beginning of each testing session, using a rotameter (InFlux OF1"S, 60-600 L/min flow, Techniquip Ltd, Taunton, England). Participants were instructed to cough through the filter so that the peak flow of each cough was measured by the pneumotachograph system and the portable device. Participants gave five strong coughs (from total lung capacity), five weak coughs (from residual volume) and five coughs of subjectively moderate strength (between strong and weak cough efforts). Participants were seated during testing. Altogether, 300 coughs were measured per test device. Devices were rotated in random order.

For the third experiment, short duration airflow bursts of consistent peak flow were mechanically generated using a 50 L pressure vessel (Medical Engineering Department, Royal Brompton

Hospital, London, England) connected to a balloon occlusion valve (Medical Engineering Department, Royal Brompton Hospital, London, England). The vessel was pressurised with compressed air to a predetermined pressure, monitored with a digital manometer (C9553 Pressure Meter, Comark, Norwich, England), at which point the occlusion valve was opened and a burst of short duration airflow released. The consistency of peak flow for these flow bursts was confirmed with five consecutive measurements with the pneumotachograph system. Vessel pressures of 5, 10, 15, 20, 30 and 40 cmH_2O were used, resulting in bursts of airflow with mean (SD) peak flows of 138 (0.4), 250 (1.3), 343 (0.8), 422 (0.8), 559 (1.6) and 684 (2.9) L/min, respectively. Five flow bursts at each flow level were discharged into each portable test device with bacterial filter.

4.3.4 Data analysis

Data were analysed using statistical software (Stata version 12.1, StataCorp LP, College Station, Texas; Microsoft Office Excel 2007, Microsoft Corporation, Redmond, WA). To quantify intra-subject variability of PECF measurements from maximal volitional coughs, coefficients of variation were calculated for each set of five consecutive PECF readings from the first experiment (standard deviation of five measurements/mean of five measurements) (Hankinson *et al*. 1998). To describe agreement between devices in the first experiment, data was plotted for visual analysis, and ICCs for comparison of individual absolute agreement were calculated (StataCorp 2013). Data from the second experiment was analysed using the Bland-Altman method (Bland & Altman 1999), comparing peak flow measurements obtained from the devices under test against the pneumotachograph. For the purpose of this analysis, the pneumotachograph system was taken as the gold standard measurement technique. To examine the influence of shorter rise time, a key characteristic distinguishing flow-time traces of cough from those of forced expiratory manoeuvres, differences between pneumotachograph and test devices were correlated with rise time. Results from the third experiment were plotted for visual analysis. Instrument repeatability for the three devices under test was expressed as the mean ± SD of five consecutive measurements.

4.4 Results

Twenty volunteers, mean (SD) age 45 (16) years, were studied, with at least one female and one male participant per age decade. Figure 9 displays each individual's PECF measurements obtained with the Assess, Mini-Wright and SpiroUSB devices. The Assess device was biased towards giving the highest PECF readings, and the Mini-Wright was biased towards giving the lowest PECF measurements. The ICC (95% CI) was 0.78 (0.47, 0.91) for comparison across all three devices; 0.72 (0.07, 0.91) for comparison between the Assess and Mini-Wright devices; 0.90 (0.77, 0.96) for comparison between the Mini-Wright and SpiroUSB devices; and 0.76 (0.23, 0.92) for comparison between the SpiroUSB and Assess devices. The median (range) coefficient of variation for five consecutive maximal voluntary coughs was 7% (1, 45).

Figure 9. Individual peak expiratory cough flow (PECF) measurements from 20 healthy volunteers, using three clinical flow measurement devices (Assess peak flow meter, Mini-Wright peak flow meter and SpiroUSB spirometer). For each device, the highest PECF value from five consecutive maximal volitional cough efforts is shown.

The mean differences and 95% limits of agreement for volunteers' PECF obtained with the pneumotachograph and test devices in series are plotted in Figure 10. Bland-Altman analysis indicated that the test devices returned on average lower PECF readings than the pneumotachograph system. Measurements of PECF were markedly lower using the Mini-Wright peak flow meter (mean (95% limits of agreement) bias 56 (-26, 138) L/min), with the difference increasing with increasing PECF (Spearman's rank correlation coefficient r_s=0.38, p<0.0001). The SpiroUSB also returned PECF readings that were consistently lower than those from the pneumotachograph system (mean (95% limits of agreement) bias 50 (-26, 125) L/min). Despite a small overall mean (95% limits of agreement) bias of 3 (-76, 82) L/min across the range, PECFs measured using the Assess device were lower than the pneumotachograph system at low PECF and higher at high PECF (r_s=-0.46, p<0.0001). Some coughs with low peak flows were not registered by the portable devices and were excluded from the Bland Altman analysis. The Mini-Wright and Assess peak flow meters did not register 15 coughs with PECF between 60 L/min (lowest mark on the devices' scale) and 118 L/min, as measured by the pneumotachograph. Thirty-four coughs with PECF from 89 to 207 L/min were not registered by the SpiroUSB spirometer.

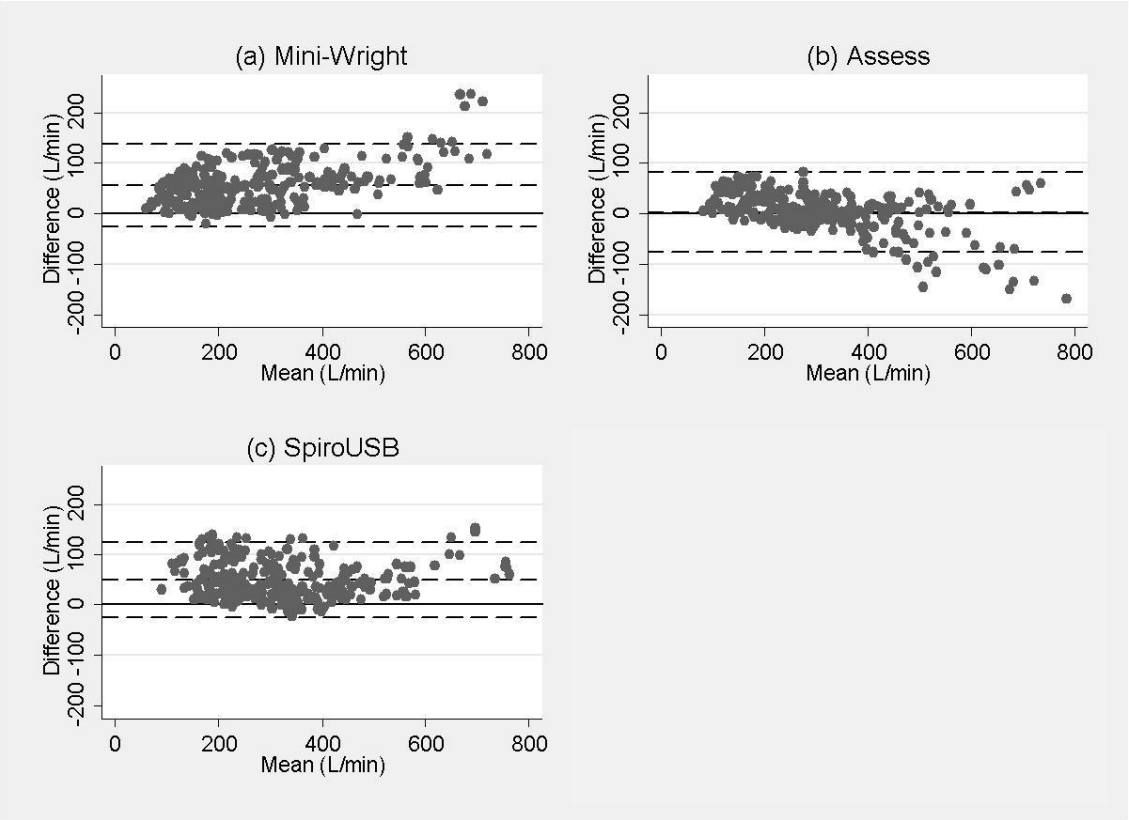


Figure 10. Bland-Altman plots of the agreement in measuring peak cough flow (L/min) between the pneumotachograph measurement system and (a) the Mini-Wright peak flow meter, (b) the Assess peak flow meter, and (c) the SpiroUSB spirometer. The difference between two measurements (pneumotachograph – second device) is plotted against the mean of two measurements. Solid lines indicate the lines of equality (no difference between measurements). Three dashed lines indicate the mean difference between measurements (bias) and the upper and lower 95% limits of agreement (bias ± 1.96SD).

Correlation analysis to examine the relationship between cough rise time (time from initiation of positive flow to peak flow) and the degree of inaccuracy indicated statistically significant weak inverse correlations for the Mini-Wright (r_s=-0.29, p<0.0001) and the Assess peak flow meter (r_s=-0.28, p<0.0001); and statistically significant moderate inverse correlations for the SpiroUSB spirometer (r_s=-0.68, p<0.0001) (Figure 11).

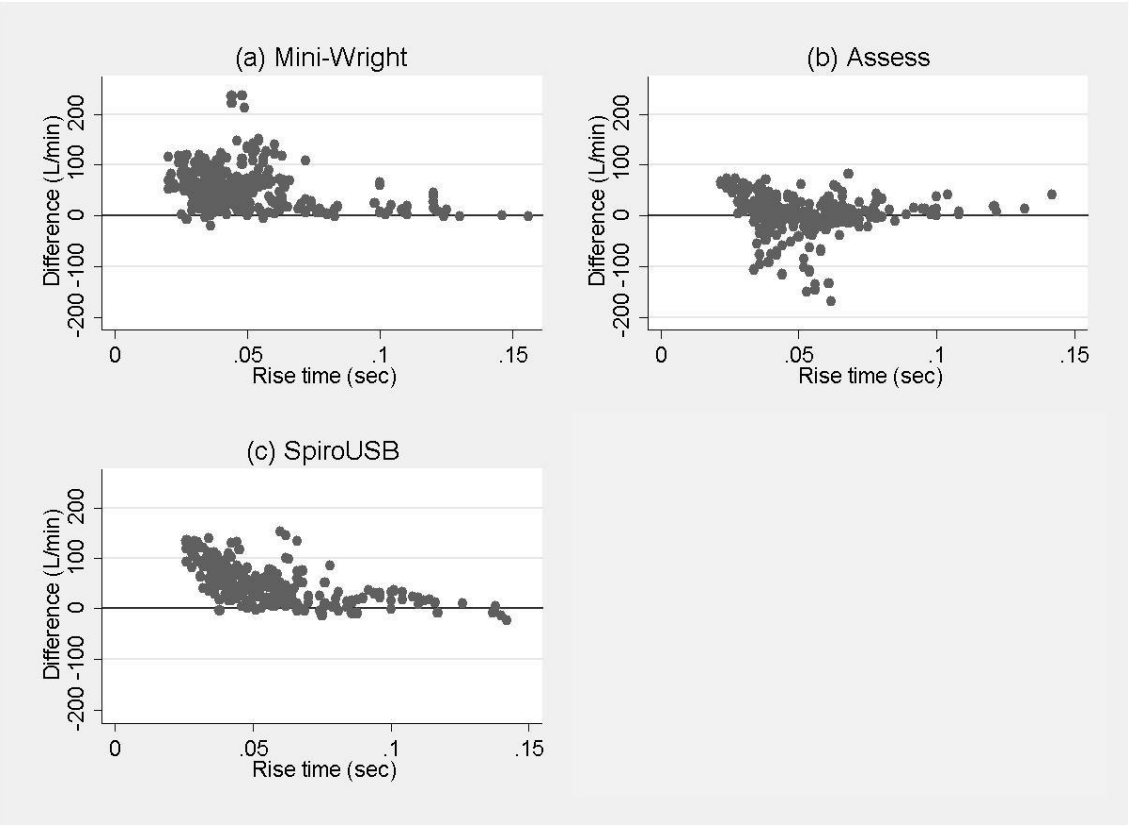


Figure 11. Coughs by healthy volunteers: The difference (pneumotachograph – alternative device) in measured peak cough flow (L/min) between pneumotachograph and (a) Mini-Wright peak flow meter, (b) Assess peak flow meter, and (c) SpiroUSB spirometer is plotted against cough rise time (sec). Solid lines indicate the lines of equality (no difference between measurements).

The results for peak flow measurements of mechanically generated flow bursts are presented in Table 22 and Figure 12. Example flow-time traces of mechanically generated flow bursts in comparison with human cough flow-time traces are given in Figure 13. The Mini-Wright device consistently returned the lowest peak flow readings. The Assess and SpiroUSB devices had good agreement up to the flow range of about 300 L/min. From 300 L/min upwards, the Assess device returned higher measurements than the SpiroUSB device.

Table 22. Peak flow measurements of mechanically generated short duration flow bursts (simulated 'coughs') using three clinical flow measurement devices.

Test device	Flow level (pressure vessel driving pressure, cmH_2O)					
	1 (5)	2 (10)	3 (15)	4 (20)	5 (30)	6 (40)
Assess (L/min)	109 ± 2	190 ± 0	253 ± 3	310 ± 0	414 ± 2	554 ± 6
Mini-Wright (L/min)	80 ± 0	131 ± 2	190 ± 0	232 ± 3	325 ± 0	390 ± 0
SpiroUSB (L/min)	110 ± 1	192 ± 2	257 ± 1	306 ± 2	384 ± 2	454 ± 3

Figures are mean ± SD

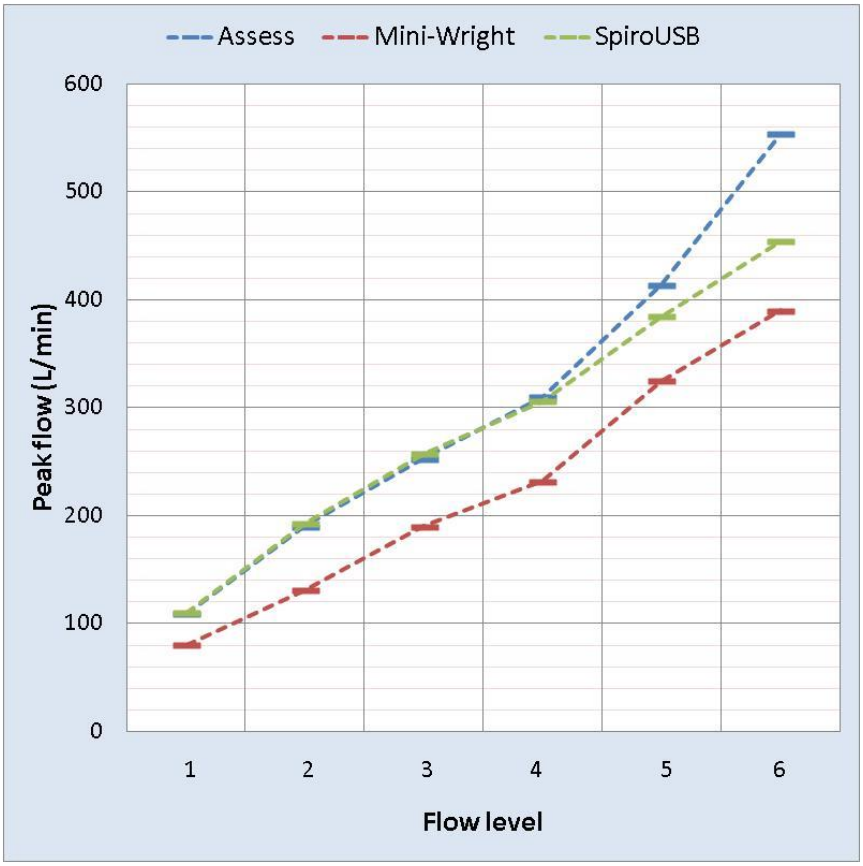


Figure 12. Peak flow measurements of mechanically generated short duration flow bursts (simulated 'coughs') obtained with three clinical flow measurement devices (Assess peak flow meter, Mini-Wright peak flow meter and SpiroUSB spirometer)

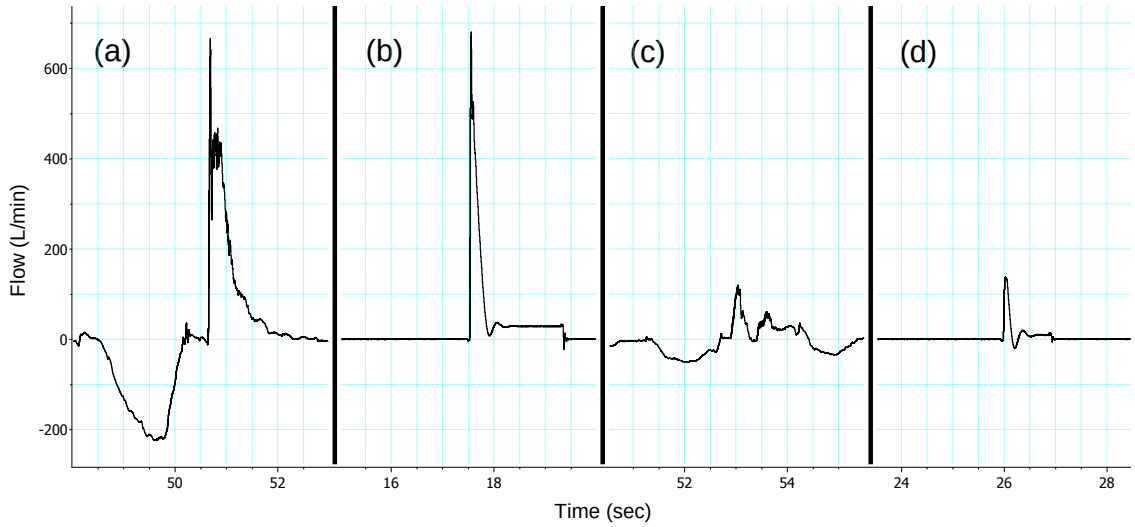


Figure 13. Examples of flow-time plots showing human coughs and mechanically generated airflow bursts of corresponding peak flow. (a) Maximally effortful voluntary cough from a healthy volunteer (peak cough flow = 666 L/min, rise time = 0.05 sec, volume expelled = 3.5 L). (b) Mechanically generated airflow burst (peak flow = 680 L/min, rise time = 0.03 sec, volume expelled = 1.6 L). (c) Maximally effortful voluntary cough from a subject with severely weakened cough following stroke (peak flow = 120 L/min, rise time = 0.14 sec, volume expelled = 0.4 L). (d) Mechanically generated airflow burst (peak flow = 138 L/min, rise time = 0.04 sec, volume expelled = 0.2 L).

4.5 Discussion

This study identified potential inaccuracy of considerable magnitude when PECF is measured using different clinical flow measurement devices. When compared to a gold standard laboratory pneumotachograph based measurement system, the three devices examined were inaccurate and returned lower PECF readings. These differences are clinically relevant when compared with the magnitude of PECF measurements in clinical populations. In addition, some low flow coughs were not registered by these devices, which impacts on their utility in very weak or severely obstructed patient populations.

The advantage of a compact, portable and practical clinical flow measurement device over a complex pneumotachograph system for the purpose of clinical practice and research is self-evident. This is particularly true for clinical populations with neuromuscular conditions, where mobility and transportation can be problematic. However, it should not be assumed that portable peak flow meters and hand-held spirometers are accurate when used for PECF measurement, as these devices are designed to measure peak flow during a forced expiratory manoeuvre. In measurements of mechanically generated airflow bursts, the portable devices under test showed good instrument repeatability, with small standard deviations at each level of flow. It could be argued that good instrument repeatability justifies the use of these devices in studies with repeated measures designs. However, the accuracy of PECF measurements becomes particularly problematic when patients are assessed against absolute thresholds. Clinical guidelines cite PECF thresholds of 160 L/min and 270 L/min to direct respiratory care of patients with neuromuscular conditions (Hull *et al*. 2012, Bott *et al*. 2009, American Thoracic Society (ATS) 2004). A scenario can be envisaged whereby the PECF measured for a patient could lie on either side of these threshold values, depending on the measurement device used. These data highlight the importance of considering which measurement device was used to measure PECF when interpreting values.

An added advantage of using a pneumotachograph system for cough flow measurement is that the flow-time trace is visualised, and further cough parameters can be derived from the trace, *i.e.* inspired pre-cough lung volume, glottis compression time, rise time and expelled lung volume. The latter two are used to compute cough volume acceleration. These parameters may provide useful information in addition to PECF. For example, Smith Hammond *et al*. (2009) found that PECF, rise time and cough volume acceleration all correlated with presence of aspiration, whereby cough volume acceleration and rise time were stronger predictors than PECF. In the study by Pitts *et al*. (2009), expiratory muscle training in individuals with Parkinson's disease lead to improvements in rise time and cough volume acceleration, but not in PECF.

The Mini-Wright and Assess peak flow meters have been used previously for PECF measurement in clinical studies (Table 21). The Assess peak flow meter (formerly Access Model 710, Health Scan Products Inc, Cedar Grove, NJ) was used in the frequently cited clinical studies by Bach and collaborators (Bach *et al*. 1997, Bach & Saporito 1996, Bach 1995), from which the current clinical recommendations for PECF thresholds are derived. The SpiroUSB spirometer has not previously been used for PECF measurement in any of the clinical studies cited here, but a similar turbine-based hand-held spirometer (Spirobank, Medical International Research, Rome, Italy) was used in the study by Fiore *et al*. (2008).

Sancho *et al*. (2004) and Silverman *et al*. (2014) have both previously examined the accuracy of different portable devices for cough flow testing using repeated maximal cough efforts. This method, however, presumes that repeated coughs are sufficiently consistent for intra-subject variability to be ignored. Although intra-subject variability may be accounted for by randomising the order of devices, and by obtaining repeated measurements within a certain range, for example three maximal coughs within 5% PECF as in the study by Sancho *et al*., intra-subject variability due to fatigue, discomfort, motivation, or practice effect remains a limitation of this method, especially with increasing number of repetitions. Also, measurements across the mid and lower range of potential values may not be assessed conveniently using maximal efforts. In the present study, the method was strengthened by connecting devices in series with a pneumotachograph and by using mechanically generated ‘coughs’ of consistent peak flow.

Using mechanically generated flow waves, simulating human expiratory flow waves with consistent peak flows, has been used previously to test performance characteristics of spirometers and peak flow meters (Miller *et al*. 2005, 2003). Such an approach allows comparison of measurement devices without the influence of intra-subject variability or bias due to in-series connection of instruments. The mechanical testing system employed in the current study produced flow bursts with peak flows and rise times within the range observed in human coughs (Sivasothy *et al*. 2001).

In cough, the time to peak flow (rise time) is shorter than during a forced expiratory manoeuvre (Miller *et al*. 2002, Sivasothy *et al*. 2001). This short rise time may be the critical characteristic causing inaccuracies in measurement, as peak flow meters and hand-held spirometers may not respond adequately to such a rapid change in signal and 'miss' the true peak. Thus, increasing inaccuracy could be expected with shorter cough rise time. The data presented here partly support this theory. There was a correlation of weak to moderate strength between cough rise time and the inaccuracy in PECF in human volunteers. Other factors contributing to differences in performance of measurement instruments may be related to the particular geometry of devices, and inertia and friction of moving parts. For spirometers, the primary purpose of use is the measurement of respiratory volumes (as opposed to flow) for the diagnosis of restrictive or obstructive lung disease. Software algorithms correct for inertia of turbine spirometers' blades to achieve optimal accuracy in volume measurements, and this may detract from absolute accuracy of peak flow measurements.

In order to advance cough flow measurement in clinical research, recommendations based on a consensus statement would be of benefit, similar to those produced for the measurement of cough frequency and reflex cough sensitivity (Morice *et al*. 2007). The frequent use of hand-held flow measurement devices for PECF assessment in clinical research demonstrates that there is a demand for technology that enables scientifically accurate, but also practical and convenient measurement of cough flow.

4.6 Conclusion

In conclusion, the three portable clinical flow measurement devices examined in our study did not accurately measure PECF. It was identified that the short rise time in cough contributes to some degree to the inaccuracies observed, although other instrument characteristics are likely to contribute to differences in instrument performance.

It is important to recognise that, depending on the measurement instrument, absolute values of PECF reported in the literature may not be directly comparable. Similarly, peak flow meters and hand-held spirometers should be used with caution when measuring PECF in clinical practice, particularly in the context of using absolute threshold values in clinical decision-making.

Instrument repeatability was shown to be good for the portable devices, which may lead to some researchers considering these devices appropriate for studies with repeated measures design. However, pneumotachograph systems have the advantage of providing further cough parameters in addition to PECF and can therefore be regarded as the preferred measurement method.

Chapter 5 Randomised controlled trial of respiratory muscle training in stroke

5.1 Introduction

Pneumonia is a relevant medical complication in acute stroke patients occurring within the first weeks of onset (Hannawi *et al*. 2013, Teramoto 2009). National Stroke Audit data for England, Wales and Northern Ireland, show PSP incidence rates of 16% and 13% in 2008 and 2010, respectively (Royal College of Physicians 2011, 2009). PSP patients have a two- to six-fold increase in risk of death (Wilson 2012, Finlayson *et al*. 2011, Koennecke *et al*. 2011, Tong *et al*. 2010, Saposnik *et al*. 2008, Sellars *et al*. 2007, Ovbiagele *et al*. 2006, Hinchey *et al*. 2005, Aslanyan *et al*. 2004, Heuschmann *et al*. 2004, Katzan *et al*. 2003, Vernino *et al*. 2003) and are three to six times more likely to have poor rehabilitation outcomes (Finlayson *et al*. 2011, Koennecke *et al*. 2011, Hong *et al*. 2008, Ovbiagele *et al*. 2006, Aslanyan *et al*. 2004). Patients with PSP are also likely to stay in the acute hospital three times longer than those without pneumonia, and require higher levels of care after hospital discharge (Wilson 2012, Finlayson *et al*. 2011, Tong *et al*. 2010, Christensen *et al*. 2009, Katzan *et al*. 2007, Ovbiagele *et al*. 2006, Hinchey *et al*. 2005).

The risk of PSP increases with increasing level of stroke severity, older age and presence of swallowing difficulty (Hoffmann *et al*. 2012, Shaheen *et al*. 2012, Finlayson *et al*. 2011, Chumbler *et al*. 2010, Lakshminarayan *et al*. 2010, Royal College of Physicians 2009, Indredavik *et al*. 2008, Sellars *et al*. 2007, Ovbiagele *et al*. 2006, Hinchey *et al*. 2005, Martino *et al*. 2005, Aslanyan *et al*. 2004, Roth *et al*. 2001, Smithard *et al*. 1996). Currently, the most widely used clinical strategy for preventing PSP is early screening for swallowing difficulties coupled with implementation of dysphagia management strategies (Hannawi *et al*. 2013, Ickenstein *et al*. 2010, Lakshminarayan *et al*. 2010, Hinchey *et al*. 2005). Other preventive approaches lack evidence and include body positioning, intensive oral hygiene, antibiotic

prophylaxis, and ACE inhibitors to improve reflex cough sensitivity (Hannawi *et* al. 2013, Teramoto 2009).

Cough (voluntary and induced) is the most immediate defence mechanism against aspiration (Fontana & Lavorini 2006). Cough production requires coordinated activation of respiratory muscles (inspiratory and expiratory) and intrinsic laryngeal muscles (Widdicombe *et al*. 2011) and is impaired in acute stroke. Studies comparing acute stroke patients with matched control subjects have shown significant reductions in respiratory muscle strength and cough flow for both voluntary and reflex cough (Zhou *et al*. 2012, Yoon *et al*. 2011, Ward *et al*. 2010, Harraf *et al*. 2008). It is not known whether respiratory muscle strength or cough flow improve with recovery in stroke patients.

RMT aims to improve respiratory performance by loading the respiratory system beyond its usual level of functioning, thereby creating a training effect. (Syabbalo 1998, Polkey *et al*. 1995, Reid & Dechman 1995, Goldstein 1993). RMT has been shown to be effective in healthy subjects, athletes and patients with cardio-respiratory conditions. A small number of studies have investigated RMT in groups with neurological conditions (Pollock *et al*. 2013) and shown that RMT can improve respiratory muscle strength in patients with degenerative neurological diseases. The clinical benefit of RMT in stroke patients remains unknown.

5.2 Aims and objectives

The aim of this pilot study was to investigate a respiratory muscle training programme in the first weeks after stroke, providing estimates on its magnitude of effect, safety, acceptability and feasibility, and informing about the value and design of a large clinical trial. The specific objectives were:

- To determine the magnitude of effect of respiratory muscle training on cough generation, respiratory muscle strength, and incidence of pneumonia

- To explore the training duration, frequency and intensity required to achieve improvement in cough flow rate and inspiratory and expiratory muscle strength
- To evaluate patient participation, acceptability of study procedures to participants, and concordance with training protocol
- To describe safety parameters and potential adverse effects of respiratory muscle training in this patient group
- To describe characteristics of those patients most likely to gain from the intervention
- To determine the relevance and feasibility of delivering respiratory muscle training to acute stroke patients in National Health Service (NHS) settings.

5.3 Methods

5.3.1 Design

This was a single-blind randomised controlled trial with three study groups. Participants were randomised to receive inspiratory muscle training, expiratory muscle training, or sham RMT. Outcome was assessed at the end of the training period (28 ± 2 days) and at 90 ± 5 days. The study was registered with the ISRCTN Register (study reference ISRCTN40298220).

5.3.2 Setting

Patients were recruited at King's College Hospital, a comprehensive stroke centre in London, UK. Participants could be discharged home or transferred to stroke rehabilitation units in local hospitals during the study period. Patients received standardised stroke rehabilitation on accredited units or from supported discharge teams at home.

5.3.3 Participants

Acute haemorrhagic or ischemic stroke patients aged 18 years and above were recruited within two weeks of stroke onset. Inclusion criteria were: NIHSS score of 5-25 with motor impairment; and ability to give informed consent and follow study procedures. Exclusion criteria were: poorly controlled hypertension (blood pressure >180/100 on three or more occasions in 24 hours); myocardial infarction, angina or acute heart failure in the preceding three months; pulmonary disease including asthma and COPD; neurological conditions other than stroke; and orthopaedic conditions adversely affecting the respiratory pump. Written informed consent was obtained prior to inclusion and the study was approved by the UK NRES (Wandsworth Research Ethics Committee, study reference 10/H0803/32).

5.3.4 Randomisation and treatment allocation

Subjects were randomly allocated after informed consent to the three trial arms on a 1:1:1 basis. The allocation sequence was concealed in sequentially numbered sealed envelopes, which contained the computer-generated randomisation codes. Block randomisation (blocks of twelve, containing four participants per study arm) was used to ensure even participant spread across the trial groups. Subjects and health care staff were blinded to treatment allocation.

5.3.5 Intervention

The training consisted of respiratory muscle strengthening undertaken daily for four weeks using the pressure threshold loading method (Reid & Samrai 1995). Participants were required to perform five sets of ten breaths with a one-minute rest in between sets, once daily. Participants were breathing in (inspiratory training) or breathing out (expiratory training) against resistance through a commercially available hand-held resistance device (Threshold IMT, Threshold PEP, Respironics, Parsippany, New Jersey). The training resistance was set at 50% of the individual's maximum inspiratory or expiratory mouth pressure for the inspiratory and

expiratory training groups, respectively. Maximal mouth pressures were re-assessed weekly and the training resistance re-adjusted to 50% of the measured maximal mouth pressure. Participants in the sham training group were also given a training device, with the resistance set to an ineffectual 10% of maximal mouth pressure. Participants were instructed in the correct training technique during the baseline session. Participants were asked to keep a daily record of training and any adverse events. Training technique and completion of the training diary were reviewed weekly.

5.3.6 Assessments and outcomes

Baseline assessments included patient demographics and stroke characteristics. Pre-morbid functional status was evaluated using the Nottingham Extended Activities of Daily Living Questionnaire (NEADL) (Gladman *et al*. 1993). Swallowing function was described according to swallow screens and clinical bedside assessments, which were conducted as part of routine acute stroke care (Appendix 2). Respiratory assessments were forced spirometry, maximal mouth pressure measurements and cough flow measurements of volitional and capsaicin-induced reflex cough. These were conducted at baseline, 28 ± 2 days (post-intervention) and 90 ± 5 days (sustainability of training effect). A detailed description of respiratory assessment methods is given in chapter 3. Incidence of pneumonia was determined from medically documented diagnosis of pneumonia or prescription of antibiotics for pneumonia. Appendix 3 details the underlying definition of pneumonia adopted for the present study.

The primary outcome measure was PECF of voluntary cough at the end of the intervention period (28 ± 2 days). Secondary outcomes were PECF of capsaicin-induced involuntary cough and maximal mouth pressures (PEmax, PImax) at 28 ± 2 days.

5.3.7 Sample size

Sample size requirements were determined using the nomogram for comparing more than two independent samples by Day and Graham (1989). The initial sample size estimate was based on an assumed group standard deviation (SD) of 50 L/min for the primary outcome measure (voluntary cough PECF), based on a cross-sectional study of acute stroke patients (Ward *et al*. 2010). A sample size of 16 subjects per group would give the study 80% power to detect a 50 L/min difference between groups at the 5% significance level. However, blinded data from the first 40 participants not divided by allocation showed PECF standard deviation of 100 L/min. A revised sample size calculation revealed that 20 participants per group would be required to detect a treatment difference of 90-100 L/min at the 5% significance level and with 80% power. Taking into account an attrition rate of 25%, the sample size of the study was increased to 78 subjects (26 in each group).

5.3.8 Statistical analysis

Descriptive and inferential statistics were used to compare baseline and outcome data between the groups. Data are presented as mean (SD), median (IQR), or frequency and percentage (%). Baseline characteristics were compared between groups using ANOVA, Kruskal-Wallis, Chi squared or Fisher's exact test as appropriate.

The main inferential analysis strategy was an "intention-to-treat" analysis of the primary and secondary outcome measures. An analysis of co-variance (ANCOVA) model was used, comparing group means of PECF of voluntary and capsaicin-induced involuntary cough, PEmax and PImax at the primary endpoint (day 28 ± 2), and adjusting for sex, age, smoking, stroke severity (NIHSS score) and training intensity. Multiple imputation was used to deal with missing values (White *et al*. 2011). In addition, an "on-treatment" analysis was also conducted, which included only those participants who remained in the study until the primary endpoint. For this, between-group comparisons of group means at the primary endpoint, mean group change

scores, and mean change scores expressed as percentage of the baseline value were conducted, using ANOVA and Kruskal-Wallis test as appropriate. Within-group comparisons from baseline to primary endpoint were conducted using Wilcoxon's signed ranks test.

Fisher's exact test was used to compare the incidence of pneumonia from baseline through week 4 between the three study groups. Sub-analyses were undertaken, comparing patients who completed the study with those who discontinued; and comparing participants with good training completion with those with poor training concordance. Training safety data was summarised descriptively and compared against pre-set safety parameters (standard safe ranges of vital parameters as used in clinical practice).

Data analyses were conducted using Stata statistical software (Stata v12.1, StataCorp, College Station, Texas).

5.4 Results

5.4.1 Recruitment and participant flow through the study

1,827 acute stroke patients were screened for eligibility, of whom 191 (10%) were eligible and 82 (4.5%) consented to participate. For the 1,636 ineligible patients, the main reasons for exclusion were NIHSS score <5 on admission (n=725, 44%), unable to give consent or follow study procedures (n=342, 21%), transfer to local hospitals in the first 72 hours (n=207, 13%), and cardiac problems in the preceding three months or respiratory and neurological conditions other than stroke (n=194, 12%). Of the 191 eligible patients, 41 (22%) declined participation in the study giving no reason; 37 (19%) thought study procedures were too demanding; 29 (15%) were not interested in research participation; and 2 (1%) were concerned about adverse effects. Of the 82 consented participants, 63 (77%) completed the primary endpoint (day 28) and 46

(56%) completed assessments at 90 days. The Consolidated Standards of Reporting Trials (CONSORT) flow diagram is shown in Figure 14.

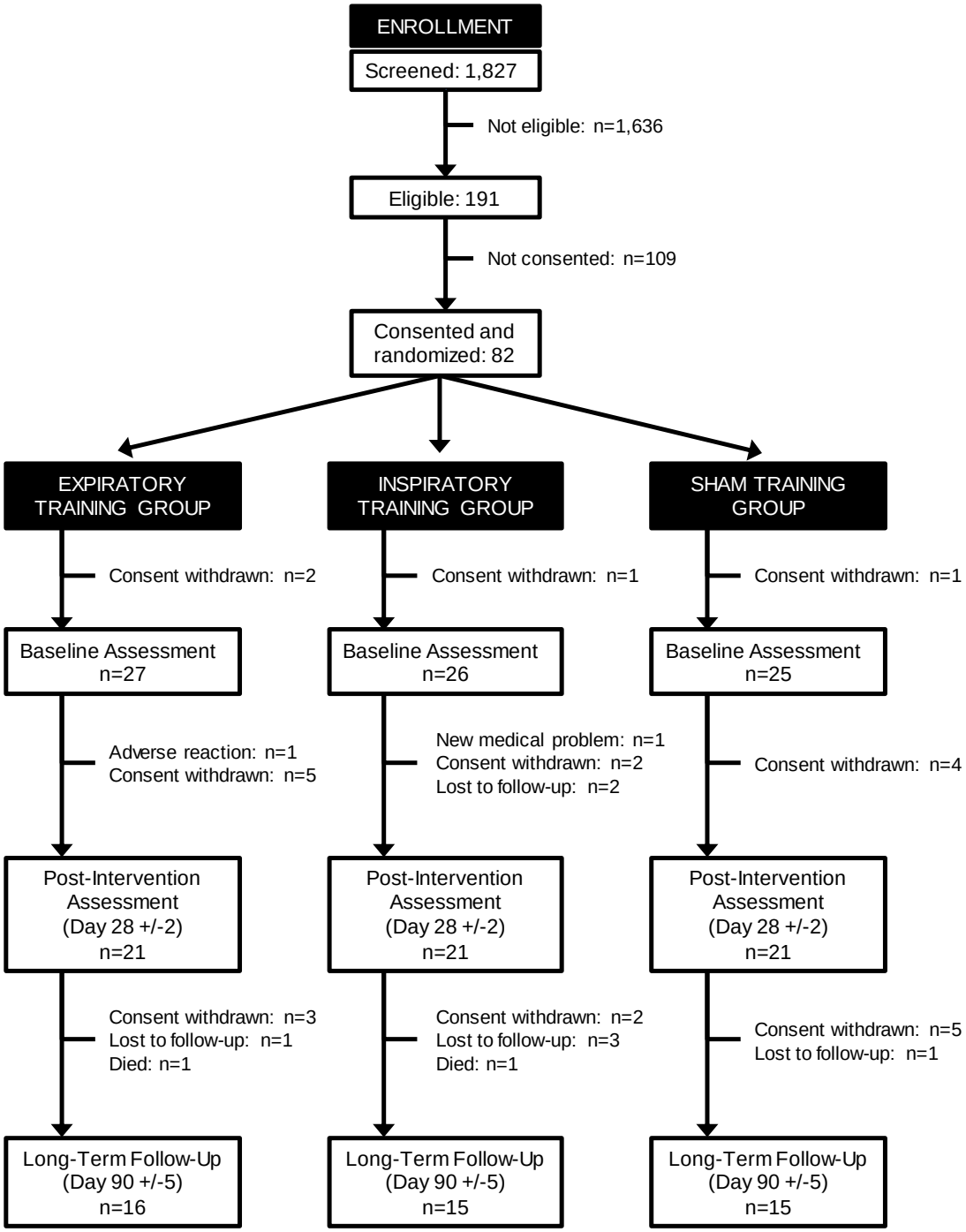


Figure 14. CONSORT flow diagram

5.4.2 Baseline characteristics

Participants were comparable across the three study groups. Patient characteristics, stroke characteristics and respiratory parameters at baseline showed no differences between the groups (Table 23).

Table 23. Baseline characteristics according to study group

	Expiratory training (n=27)	Inspiratory training (n=26)	Sham training (n=25)	p-value
Age (years)	65.7 (15.4)	62.5 (14.6)	65.1 (13.9)	0.702
Males	14 (52%)	17 (65%)	16 (64%)	0.541
NIHSS score (median, IQR)	7 (5, 12)	8 (6, 11)	8 (6, 12)	0.645
Pre-morbid NEADL score (median, IQR)	61 (45, 63)	59 (48, 63)	57 (46, 63)	0.875
Stroke Type				
Ischemic	22 (82%)	23 (88%)	24 (96%)	0.308
Haemorrhagic	5 (18%)	3 (12%)	1 (4%)	0.308
Stroke Side				
Left	10 (37%)	8 (31%)	11 (44%)	0.689
Right	17 (63%)	17 (65%)	14 (56%)	0.689
Bilateral	-	1 (4%)	-	0.689
Stroke Site				
Cortical	12 (44%)	10 (38%)	11 (44%)	0.273
Subcortical	14 (52%)	12 (46%)	8 (32%)	0.273
Brainstem/cerebellar	1 (4%)	4 (15%)	6 (24%)	0.273

Table 23. continued

Current smoker	6 (22%)	5 (19%)	9 (36%)	0.344
Unsafe swallow on BSA	13 (48%)	10 (38%)	12 (48%)	0.723
Forced spirometry				
FVC (L)	2.2 (1.0)	2.1 (0.8)	2.4 (1.0)	0.437
FEV_1 (L)	1.7 (0.9)	1.8 (0.7)	2.0 (0.9)	0.394
PEF (L/min)	246 (145)	238 (130)	276 (149)	0.596
Maximal voluntary cough				
PICF (L/min)	123 (59)	125 (66)	149 (85)	0.272
PECF (L/min)	471 (218)	465 (307)	516 (278)	0.644
CVI (L)	1.7 (0.9)	1.4 (0.7)	1.6 (0.6)	0.455
CVE (L)	1.3 (0.8)	1.3 (0.7)	1.5 (0.7)	0.560
CVAC (L/s/s)	160 (92)	171 (132)	189 (128)	0.694
GCT (s)	0.24 (0.1)	0.28 (0.2)	0.22 (0.1)	0.289

Table 23. continued

Capsaicin-induced involuntary cough				
PICF (L/min)	91 (46)	88 (49)	83 (35)	0.884
PECF (L/min)	279 (112)	313 (143)	272 (103)	0.823
CVI (L)	1.3 (0.6)	1.1 (0.6)	1.1 (0.5)	0.428
CVE (L)	0.7 (0.3)	0.7 (0.4)	0.6 (0.3)	0.994
CVAC (L/s/s)	105 (50)	127 (62)	117 (42)	0.358
GCT (s)	0.18 (0.1)	0.24 (0.14)	0.21 (0.1)	0.341
Maximal mouth pressures				
PEmax (cmH_2O)	62 (34)	56 (34)	64 (34)	0.636
PImax (cmH_2O)	39 (32)	42 (27)	45 (27)	0.690

Figures are mean (SD) and frequency (%), unless stated otherwise

p-values were calculated using the appropriate parametric (ANOVA) or non-parametric (Kruskal-Wallis test) statistical significance test for continuous data, and Chi squared or Fisher's exact test for categorical data

BSA, bedside swallowing assessment; CVAC, cough volume acceleration; CVE, cough volume expired; CVI, cough volume inspired; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; GCT, glottis compression time; NEADL, Nottingham Extended Activities of Daily Living questionnaire; PECF, peak expiratory cough flow; PEF, peak expiratory flow; PEmax, maximal expiratory mouth pressure; PICF, peak inspiratory cough flow; PImax, maximal inspiratory mouth pressure

5.4.3 Intention-to-treat analysis

Table 24 summarises all available data at the study time points for cough flow and respiratory muscle strength outcomes. Figure 15 displays group means over time.

Table 24. Intention-to-treat analysis: cough flow and maximal mouth pressure outcomes at the study time points

	Baseline	Primary endpoint (day 28±2)	Long-term follow-up (day 90±5)	p-values [a]
Expiratory training (n=27)				
Voluntary cough PECF (L/min)	471 (218)	556 (274)	578 (223)	0.135
Involuntary cough PECF (L/min)	279 (112)	317 (129)	324 (149)	0.469
PEmax (cmH_2O)	62 (34)	80 (31)	87 (30)	0.003
PImax (cmH_2O)	39 (32)	55 (33)	64 (32)	0.002
Inspiratory training (n=26)				
Voluntary cough PECF (L/min)	465 (307)	553 (366)	517 (393)	0.010
Involuntary cough PECF (L/min)	313 (143)	301 (182)	247 (121)	0.586
PEmax (cmH_2O)	56 (34)	70 (34)	81 (33)	<0.001
PImax (cmH_2O)	42 (27)	60 (34)	63 (38)	<0.001
Sham training (n=25)				
Voluntary cough PECF (L/min)	516 (278)	656 (321)	504 (202)	0.016
Involuntary cough PECF (L/min)	272 (103)	310 (104)	295 (102)	0.124
PEmax (cmH_2O)	64 (34)	80 (40)	81 (38)	0.007
PImax (cmH_2O)	45 (27)	65 (30)	64 (35)	<0.001

Figures are mean (SD)

[a] p-values are for within-group comparisons from baseline to primary endpoint and were calculated using the Wilcoxon's signed ranks test with no adjustment for multiple testing

PECF, peak expiratory cough flow; PEmax, maximal expiratory mouth pressure; PImax, maximal inspiratory mouth pressure

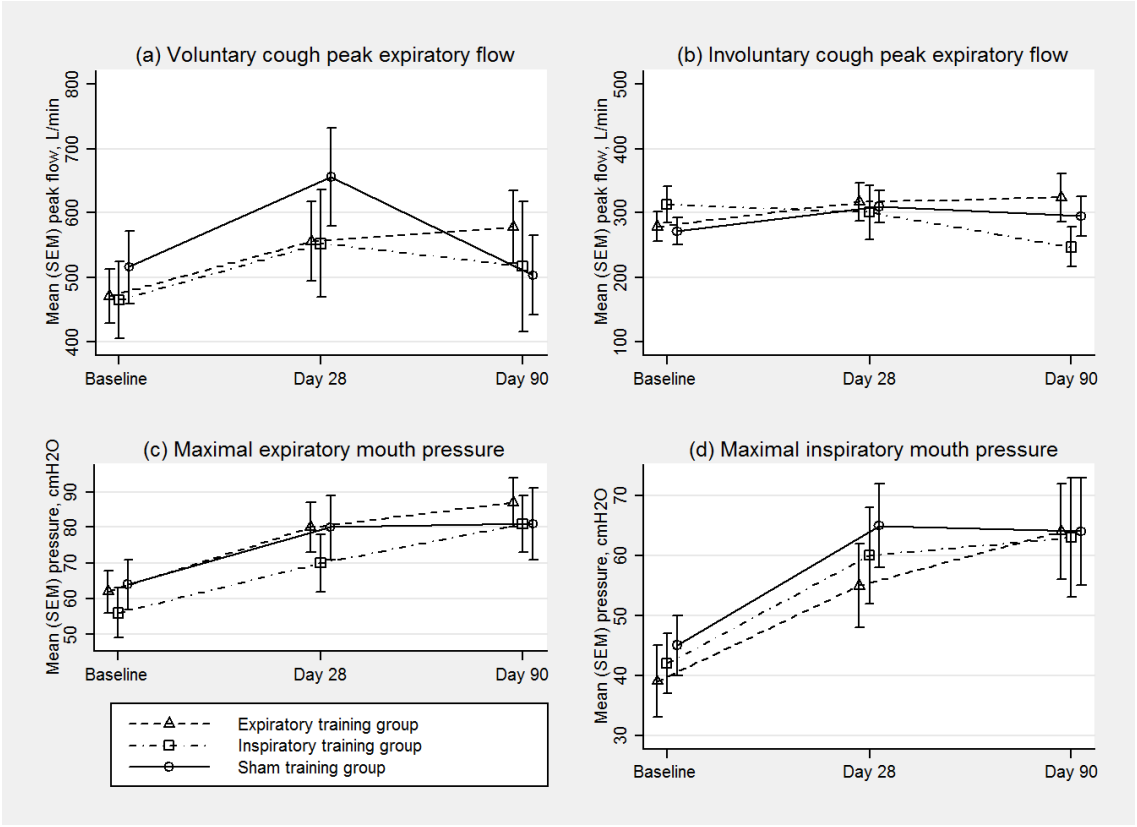


Figure 15. Cough flow and maximal mouth pressure outcomes at the study time points by study group. Error bars represent standard error of the mean (SEM).

Intention-to-treat analyses for cough flow and maximal mouth pressure outcomes were conducted using an ANCOVA model. Each intervention group was compared against the sham training group at the primary endpoint. Missing data were imputed through multiple imputation, using predictive model-based imputation (White *et al*. 2011, StataCorp 2011).

The missing data structure was assessed. Logistic regression was used to assess for potential predictors for “missingness”. All predictor variables for “missingness” with p-value <0.1 were included in the ANCOVA analysis model, thereby creating missing at random (MAR) conditions.

Multiple imputations were then conducted following the usual step-wise process (imputation and analysis). Twenty multiply imputed datasets were generated per outcome (StataCorp 2011).

Linear regression was used to generate multiply imputed datasets. All covariates used in the eventual ANCOVA model were also included in the generation of imputed values. Summary statistics for selected imputations were compared and no obvious abnormalities were noted.

In the analysis step, which combines complete-data analyses and pooling of complete-data analyses, ANCOVA was conducted using pre-determined independent variables (baseline level of the outcome variable, study group, sex, age, baseline level of peak expiratory flow, stroke severity at admission, and training intensity). Outcome variables were transformed using the natural logarithm function to meet model assumptions of normal distribution. Results are summarized in Table 25, with regression coefficients, standard errors and 95% confidence intervals presented at the transformed level of the outcome variables.

ANCOVA analysis showed no statistically significant treatment effect of either expiratory or inspiratory training when compared with sham RMT. One single comparison (involuntary PECF in the inspiratory training group) showed a statistically significant p-value. However, with adjustment for multiple testing this p-value becomes non-significant.

Table 25. Intention-to-treat analysis comparing intervention groups to the sham training group, using analysis of co-variance (ANCOVA) and multiple imputation through predictive model-based imputation for missing data. To meet model assumptions, data were transformed using the natural logarithm function (ln). Regression coefficients, standard errors and confidence intervals are presented at the level of the transformed scale.

Outcome parameter	Model statistics	Group in comparison with sham training	Coef.	SE	t-statistic	p-value	95% CI
Voluntary cough PECF (ln(L/min))	n=78 (55 complete, 23 imputed)	Expiratory training	-0.091	0.078	-1.17	0.246	-0.247, 0.064
	Imputations: 20 Average RVI: 0.5183 Largest FMI: 0.5026 Complete DF: 69 DF min: 24.60 DF avg: 37.33 DF max: 53.41 F (8, 61.3) = 32.28	Inspiratory training	0.050	0.086	0.58	0.562	-0.123, 0.223
Involuntary cough PECF (ln(L/min))	n=74 (54 complete, 20 imputed)	Expiratory training	-0.113	0.100	-1.14	0.263	-0.316, 0.089
	Imputations: 20 Average RVI: 0.5319 Largest FMI: 0.5666 Complete DF: 65 DF min: 20.10 DF avg: 34.27 DF max: 43.99 F (8, 57.3) = 11.56	Inspiratory training	-0.194	0.094	-2.05	0.046	-0.384, -.003

Table 25. continued

PEmax ($ln(cmH_2O)$)	n=78 (61 complete, 17 imputed)	Expiratory training	0.038	0.099	0.38	0.706	-0.162, 0.237
	Imputations: 20 Average RVI: 0.4212 Largest FMI: 0.4108 Complete DF: 70 DF min: 31.07 DF avg: 41.14 DF max: 48.45 F (8, 63.6) = 14.76	Inspiratory training	0.085	0.104	0.82	0.419	-0.125, 0.295
PImax ($ln(cmH_2O)$)	n=78 (61 complete, 17 imputed)	Expiratory training	-0.144	0.091	-1.58	0.120	-0.327, 0.039
	Imputations: 20 Average RVI: 0.4218 Largest FMI: 0.5518 Complete DF: 69 DF min: 21.74 DF avg: 41.19 DF max: 56.77 F (8, 62.5) = 26.87	Inspiratory training	0.070	0.096	0.73	0.470	-0.123, 0.263

PECF, peak expiratory cough flow; PEmax, maximal expiratory mouth pressure; PImax, maximal inspiratory mouth pressure

5.4.4 On-treatment analysis

On-treatment (complete case) analyses were conducted for participants who remained in the study from baseline to primary endpoint (day 28 ± 2). Within-group and between-group comparisons are shown in Tables 26 and 27, respectively. There were no statistically significant differences in between-group comparisons of outcome parameters expressed as group means at the primary endpoint, group mean changes from baseline to primary endpoint, and change as percentage of the baseline value. Within-group comparison showed statistically significant improvements for all outcomes but PECF of involuntary cough. For the entire sample, improvements in PECF of voluntary cough, PEmax and PImax were highly significant with p-values of 0.0002, <0.0001 and <0.0001, respectively.

Table 26. On-treatment analysis: within-group comparison of change (day 28 – baseline) in cough flow and respiratory muscle strength from baseline to primary endpoint

		Group		
	All patients	Expiratory training (n=21)	Inspiratory training (n=21)	Sham training (n=21)
Change in voluntary cough PECF (L/min)	74 (150)	49 (121)	91 (184)	84 (146)
p-value [a]	0.0002	0.135	0.010	0.016
Change in involuntary cough PECF (L/min)	14 (95)	17 (83)	-4 (121)	32 (76)
p-value [a]	0.328	0.469	0.586	0.124
Change in PEmax (cmH_2O)	15 (18)	12 (15)	20 (20)	12 (18)
p-value [a]	<0.0001	0.003	0.0006	0.0071
Change in PImax (cmH_2O)	14 (16)	10 (12)	18 (20)	14 (15)
p-value [a]	<0.0001	0.0018	0.0004	0.0002

Figures are mean (SD)

[a] p-values were calculated using the Wilcoxon's signed ranks test with no adjustment for multiple testing

PECF, peak expiratory cough flow; PEmax, maximal expiratory mouth pressure; PImax, maximal inspiratory mouth pressure

Table 27. On-treatment analysis: between-group comparison of group means at the primary endpoint, group mean changes (day 28 – baseline) and group mean changes expressed as percentage of the baseline measurement, for cough flow and maximal mouth pressures

	Expiratory training (n=21)	Inspiratory training (n=21)	Sham training (n=21)	p-value [a]
Voluntary cough				
PECF at day 28 (L/min)	556 (274)	552 (366)	656 (321)	0.47
Change in PECF (day 28 – baseline, L/min)	49 (121)	91 (184)	84 (146)	0.46
Percentage change from baseline (%)	20 (22)	32 (34)	23 (25)	0.37
Reflex cough				
PECF at day 28 (L/min)	317 (129)	301 (182)	310 (104)	0.42
Change in PECF (day 28 – baseline, L/min)	17 (83)	-4 (121)	32 (76)	0.41
Percentage change from baseline (%)	9 (26)	-2 (33)	18 (29)	0.14
PEmax				
PEmax at day 28 (cmH_2O)	80 (31)	70 (34)	80 (40)	0.59
Change in PEmax (day 28 – baseline, cmH_2O)	12 (15)	20 (20)	12 (18)	0.35
Percentage change from baseline (%)	32 (46)	47 (52)	26 (41)	0.37
PImax				
PImax at day 28 (cmH_2O)	55 (33)	60 (34)	65 (30)	0.47
Change in PImax (day 28 – baseline, cmH_2O)	10 (12)	18 (20)	14 (15)	0.30
Percentage change from baseline (%)	38 (50)	55 (56)	34 (42)	0.37

Figures are mean (SD), [a] p-values were calculated using ANOVA or Kruskal-Wallis test as appropriate; PECF, peak expiratory cough flow; PEmax, maximal expiratory mouth pressure; PImax, maximal inspiratory mouth pressure

5.4.5 Incidence of pneumonia

Table 28 shows the cumulative incidence of pneumonia by day 28 for all participants who remained in the study until the primary endpoint, and there was no statistically significant difference between the study groups. As participants who discontinued the study were not followed up for the incidence of pneumonia, intention-to-treat analysis was conducted through a sensitivity analysis of four extreme possible outcomes: (a) none of the participants who dropped out developed pneumonia; (b) all participants who dropped out developed pneumonia; (c) none of the participants who dropped out in the sham training group developed pneumonia, and all of those who dropped out in the intervention groups did (negative effect of the interventions on pneumonia rates); and (d) all of the participants who dropped out in the sham training group developed pneumonia, and none of those who dropped out in the intervention groups did (positive effect of the interventions on pneumonia rates). These scenarios are summarised in Table 29. In none of the scenarios the p-value is statistically significant.

Table 28. Number of participants developing pneumonia according to study group, only including participants who remained in the study until the primary endpoint. Fisher's exact test shows no statistically significant difference between the study groups (p =1.0).

	No Pneumonia by day 28	Pneumonia by day 28	Total
Expiratory training	18 (86%)	3 (14%)	21
Inspiratory training	19 (90%)	2 (10%)	21
Sham training	18 (86%)	3 (14%)	21
Total	55 (87%)	8 (13%)	63

Figures are number of participants (percentage of row total)

Table 29. Sensitivity analysis of cumulative pneumonia incidence by day 28. Four extreme scenarios are compared.

Scenario (a) none of the participants who dropped out developed pneumonia, p=0.902

	No Pneumonia by day 28	Pneumonia by day 28	Total
Expiratory training	26 (90%)	3 (10%)	29
Inspiratory training	25 (93%)	2 (7%)	27
Sham training	23 (88%)	3 (12%)	26
Total	74 (90%)	8 (10%)	82

Scenario (b) all participants who dropped out developed pneumonia, p=0.836

	No Pneumonia by day 28	Pneumonia by day 28	Total
Expiratory training	18 (62%)	11 (38%)	29
Inspiratory training	19 (70%)	8 (30%)	27
Sham training	18 (69%)	8 (31%)	26
Total	55 (67%)	27 (33%)	82

Scenario (c) none of the participants who dropped out from the sham training group developed pneumonia, and all participants who dropped out from the intervention groups did, p=0.070

	No Pneumonia by day 28	Pneumonia by day 28	Total
Expiratory training	18 (62%)	11 (38%)	29
Inspiratory training	19 (70%)	8 (30%)	27
Sham training	23 (88%)	3 (12%)	26
Total	60 (73%)	22 (27%)	82

Table 29. continued

Scenario (d) all participants who dropped out from the sham training group developed pneumonia, and none of the participants who dropped out from the intervention groups did, p=0.060

	No Pneumonia by day 28	Pneumonia by day 28	Total
Expiratory training	26 (90%)	3 (10%)	29
Inspiratory training	25 (93%)	2 (7%)	27
Sham training	18 (69%)	8 (31%)	26
Total	69 (84%)	13 (16%)	82

Figures are number of participants (percentage of row total)

p-values were calculated using Fisher's exact test

5.4.6 Treatment concordance and impact on outcomes

Table 30 presents details of training frequency by study group. There was wide variation in how often participants trained, from those who completed the training as prescribed (28 days, 140 sets, 1,400 breaths) to those who trained minimally (only when prompted by the investigator during follow-up visits). Across the entire sample, the median (IQR) number of training days was 17 (6, 24), and the median (IQR) number of training breaths was 803 (250, 1,150).

Baseline characteristics were compared for those participants who had high training concordance (who trained half or more of the prescribed training breaths, n=42) and low training concordance (who trained less than half of the prescribed training breaths, n=21). Participants who trained more were generally more able from the start. Although both groups had median NIHSS scores of 8, those who trained more were younger (mean age 62 *versus* 68 years), had somewhat better pre-morbid function (median NEADL score 63 *versus* 53), and better respiratory parameters throughout (for example mean PECF of voluntary cough 530 L/min

versus 394 L/min). Cough flow and respiratory muscle strength outcomes were compared for participants who trained more and participants who trained less (Table 31).

Table 30. Training concordance by study group

	Expiratory training (n=27)	Inspiratory training (n=26)	Sham training (n=25)	p-value
Days from onset of stroke to baseline assessment	7 (4, 11)	6 (3, 9)	7 (3, 10)	0.577
Days trained [a]	17 (6, 27)	17 (9, 22)	16 (5, 25)	0.871
Breaths trained [b]	806 (300, 1,303)	799 (260, 1,050)	800 (230, 1,215)	0.583
Training completion (mean (SD) percentage of maximum prescribed)	56 (36)	48 (32)	52 (37)	0.664

Figures are median (IQR) unless stated otherwise

p-values were calculated using ANOVA and Kruskal-Wallis test as appropriate

[a] maximum prescribed: 28 days

[b] maximum prescribed: 1,400 breaths

Table 31. Group means and mean change for cough flow and respiratory muscle strength outcomes in participants who trained half or more (700+ breaths) of the prescribed repetitions compared with participants who trained less than half (<700) of the prescribed repetitions

Outcome	Trained 700+ breaths (n=42)	Trained <700 breaths (n=21)	p-value
Voluntary cough			
PECF at day 28 (L/min)	631 (325)	473 (282)	0.099
Change in PECF (day 28 – baseline, L/min)	92 (166)	28 (90)	0.076
Involuntary cough			
PECF at day 28 (L/min)	315 (154)	295 (103)	0.787
Change in PECF (day 28 – baseline, L/min)	26 (97)	-14 (87)	0.185
Maximal mouth pressures			
PEmax at day 28 (cmH_2O)	85 (35)	59 (29)	0.003
Change in PEmax (day 28 – baseline, cmH_2O)	18 (20)	9 (13)	0.102
PImax at day 28 (cmH_2O)	68 (34)	44 (22)	0.006
Change in PImax (day 28 – baseline, cmH_2O)	15 (18)	12 (11)	0.732

Figures are mean (SD)

PECF, peak expiratory cough flow; PEmax, maximal expiratory mouth pressure; PImax, maximal inspiratory mouth pressure

p-values were calculated using the Mann-Whitney U test with no adjustment for multiple testing

5.4.7 Training safety and adverse events

Vital parameters before and after respiratory training for the entire study sample are listed in Table 32. Sample means of blood pressure, heart rate and oxygen saturation remained stable before and after respiratory muscle training, although individual participants did show fluctuations in measurements. Measurements were compared with standard safe reference

ranges for these parameters, as used in clinical practice (safe range for systolic blood pressure 110-130 mmHg; diastolic blood pressure <105 mmHg; heart rate 60-120 bpm; oxygen saturation >90%). Reports of subjective symptoms were recorded, in particular of headache, chest pain, or overall strain.

Altogether, vital parameters were monitored in 254 training session. Where vital parameters were outside the safe reference ranges, they were within the individual medically accepted range for the person and stable before and after training, and participants did not report any training-related subjective adverse symptoms.

In one single case, an increase in blood pressure from 140 mmHg before training to 187 mmHg after training (EMT) was observed. This participant also reported subjective strain and discomfort in the region of both temples. Blood pressure and subjective symptoms resolved within minutes of discontinuing training. This participant was withdrawn from the study, and the episode was recorded as a significant adverse event related to the study intervention.

Other observations recorded as minor adverse events and unrelated to study procedures were headache and general fatigue. Observations recorded as minor adverse events and related to study procedures were: fatigue after training (within acceptable range for an acute exercise intervention); and light-headedness after training (resolved by increasing the number of pauses between training breaths).

Table 32. Vital parameters taken immediately before and after training. Parameters were measured at baseline and in weekly investigator visits.

	Systolic blood pressure (mmHg)		Diastolic blood pressure (mmHg)		Heart rate (bpm)		Oxygen saturation (%)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Baseline (n=78)	132 (19)	132 (20)	81 (13)	82 (13)	76 (14)	75 (13)	96 (2)	96 (2)
Day 7 (n=66)	125 (19)	124 (16)	80 (11)	81 (12)	76 (12)	76 (13)	97 (2)	97 (2)
Day 14 (n=59)	126 (17)	124 (15)	79 (13)	78 (12)	74 (13)	76 (13)	96 (2)	97 (2)
Day 21 (n=51)	128 (17)	129 (16)	81 (11)	83 (10)	74 (13)	73 (14)	96 (2)	96 (2)

Figures are mean (SD)

5.4.8 Between-group comparison of area under the curve for respiratory muscle strength outcomes

Weekly measurements of maximal mouth pressures were analysed using area under the curve (AUC) and time of maximum (Tmax) as summary measures. The rationale was that groups may not differ when compared with respect to their change from baseline to primary endpoint (day 28±2); but that the improvement in maximal mouth pressures seen across all groups may have set in earlier in the intervention groups, thereby providing improved respiratory function and protection from aspiration earlier than in the control group. Maximal mouth pressure measurements were made weekly during the intervention period.

All participants who remained in the study until the primary endpoint were included in this analysis (n=63). Forty-five missing observations (all follow-up sessions at weeks one, two or three) were imputed by using the lower of the preceding or following available observation, thereby giving a conservative method of single imputation. The results are presented in Table

33. There were minor differences between the groups in AUC and Tmax, none of which were statistically significant.

Table 33. Between-group comparison of area under the curve (AUC) and time to maximum (Tmax) for expiratory and inspiratory mouth pressures

	Group			p-value	
	Sham training	Expiratory training	Inspiratory training	ANOVA	Kruskal-Wallis
PEmax					
AUC	294 (144)	297 (120)	253 (140)	0.51	0.26
Tmax (week)	2.3 (1.5)	2.8 (1.5)	2.8 (1.3)	0.38	0.34
PImax					
AUC	229 (114)	199 (126)	206 (125)	0.70	0.53
Tmax (week)	3.2 (1.2)	3.1 (1.4)	3.0 (1.3)	0.89	0.80

Figures are mean (standard deviation) and number of observations

ANOVA, analysis of variance; AUC, area under the curve; PEmax, maximal expiratory mouth pressure; PImax, maximal inspiratory mouth pressure; Tmax, time of maximum

5.4.9 Correlation between training frequency, change in cough flow and change in respiratory muscle strength

Correlation analysis (Spearman's rank correlation coefficient) was conducted to explore in how far training frequency (total number of training breaths) was correlated with change in cough flow and respiratory muscle strength. Correlation was examined for absolute change in outcome and for percentage change from baseline. The findings are summarised in Table 34.

The correlations observed are of weak to moderate strength, for both absolute change on outcome and percentage change from baseline. The highest correlation is with voluntary cough PECF in the sham group (r_s=0.42), but this is not statistically significant. The only statistically significant correlation is with change in voluntary cough PECF for the entire sample (r_s=0.30; p=0.021).

Table 34. Correlation between training frequency (total number of training breaths) and outcomes

		Group		
	Sample	Sham training	Expiratory training	Inspiratory training
Change in voluntary cough PECF (day 28 – baseline, L/min)	r_s=0.30, n=57, p=0.021	r_s=0.42, n=18, p=0.088	r_s=0.23, n=20, p=0.334	r_s=0.36, n=19, p=0.131
Voluntary cough PECF percentage change from baseline (%)	r_s=0.13, n=57, p=0.320	r_s=0.42, n=18, p=0.083	r_s=-0.27, n=20, p=0.243	r_s=0.25, n=19, p=0.297
Change in involuntary cough PECF (day 28 – baseline, L/min)	r_s=0.08, n=54, p=0.567	r_s=0.24, n=17, p=0.343	r_s=-0.08, n=19, p=0.758	r_s=0.08, n=18, p=0.741
Involuntary cough PECF percentage change from baseline (%)	r_s=0.12, n=54, p=0.404	r_s=0.18, n=17, p=0.486	r_s=0.02, n=19, p=0.932	r_s=0.12, n=18, p=0.642
Change in PEmax (day 28 – baseline, cmH_2O)	r_s=0.14, n=63, p=0.280	r_s=0.33, n=21, p=0.146	r_s=0.25, n=21, p=0.271	r_s=-0.07, n=21, p=0.750
PEmax percentage change from baseline (%)	r_s=0.01, n=63, p=0.965	r_s=0.12, n=21, p=0.604	r_s=0.13, n=21, p=0.570	r_s=-0.16, n=21, p=0.487
Change in PImax (day 28 – baseline, cmH_2O)	r_s=-0.06, n=63, p=0.627	r_s=-0.11, n=21, p=0.621	r_s=-0.06, n=21, p=0.807	r_s=0.26, n=21, p=0.250
PImax percentage change from baseline (%)	r_s=-0.18, n=63, p=0.164	r_s=-0.14, n=21, p=0.554	r_s=-0.14, n=21, p=0.551	r_s=-0.09, n=21, p=0.693

Figures are Spearman's rank correlation coefficient, number of observations, and p-values (with no adjustment for multiple testing)

PECF, peak expiratory cough flow; PEmax, maximal expiratory mouth pressure; PImax, maximal inspiratory mouth pressure

5.4.10 Pooled analysis of outcome respiratory muscle strength including previous research

In order to increase the power of analysis, data from the present study was pooled with findings from two previous randomised controlled trials of RMT in subacute and chronic stroke patients, in which maximal mouth pressure outcomes (PImax, PEmax) were reported (Britto *et al*. 2011, Sutbeyaz *et al*. 2010). These findings were combined in a random effects meta-analysis using non-standardised effect sizes. Data were pooled according to two assumptions: (a) assuming that both inspiratory (IMT) and expiratory (EMT) muscle training could lead to improvements in either PImax, PEmax or both (Figure 16); and (b) assuming that the outcome should correspond with the direction of training, combining studies of inspiratory muscle training (IMT) for outcome PImax, and studies of expiratory muscle training (EMT) for outcome PEmax (Figure 17).

These pooled analyses increased the overall sample size. An overall trend towards improved respiratory muscle strength with RMT is shown, more so for outcome PImax; however, this is not statistically significant and the intervention effect can be described as modest in absolute terms

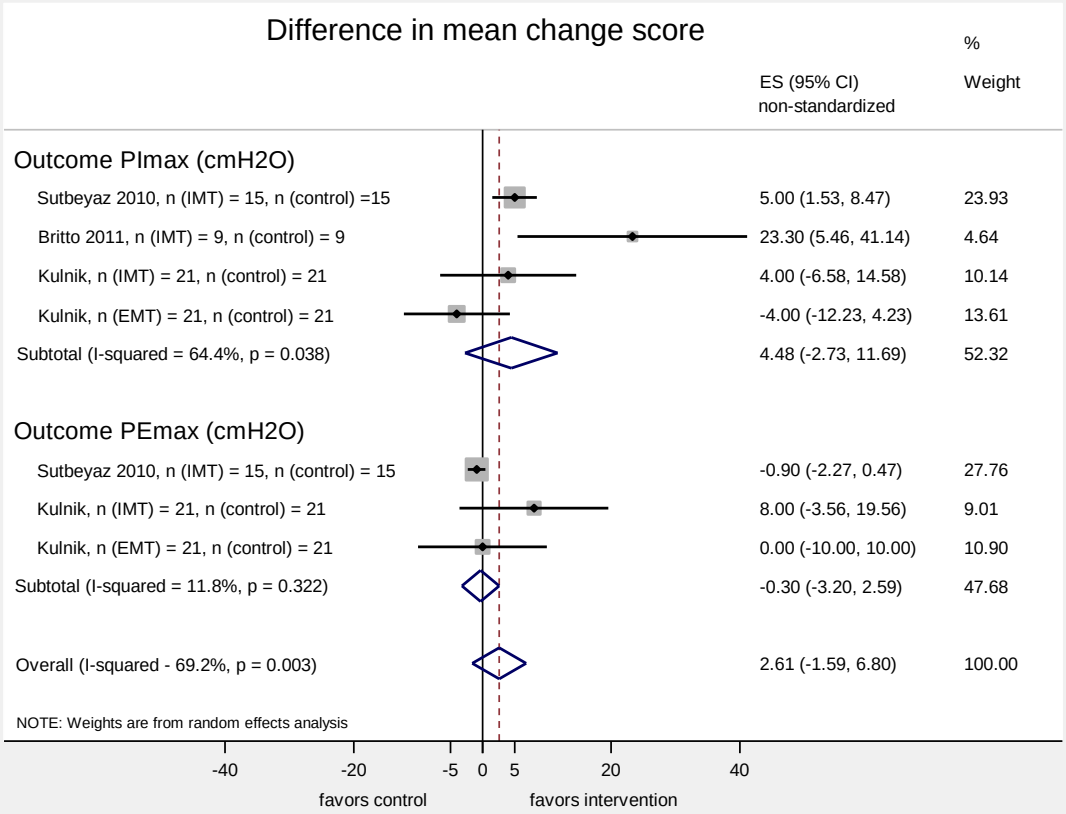


Figure 16. Random effects meta-analysis, assuming that both inspiratory (IMT) and expiratory (EMT) muscle training could lead to improvements in either PImax, PEmax, or both

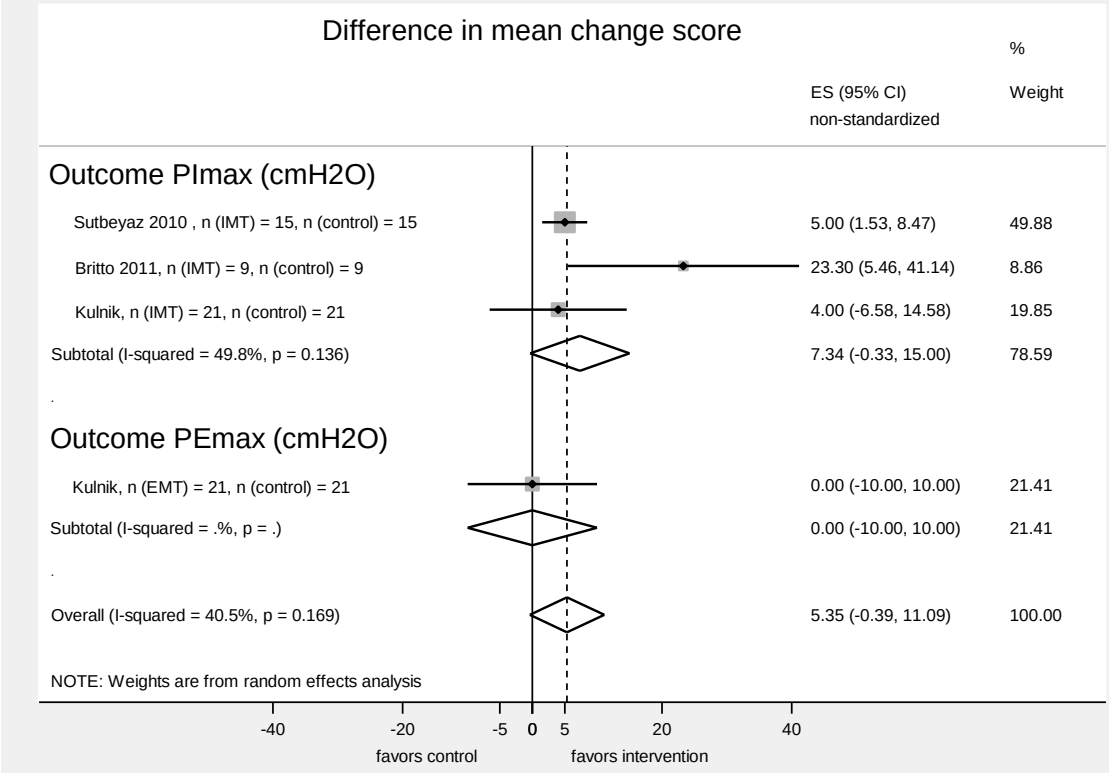


Figure 17. Random effects meta-analysis, combining studies of inspiratory muscle training (IMT) for outcome PImax, and studies of expiratory muscle training (EMT) for outcome PEmax.

5.5 Discussion

This study showed that peak expiratory flow of voluntary cough and maximal inspiratory and expiratory mouth pressures improved in the first 4 weeks after acute stroke onset, but there were no changes in peak expiratory flow of involuntary cough. A standardised programme of inspiratory or expiratory RMT delivered for 4 weeks did not significantly add to the extent or speed of recovery of respiratory muscle strength or cough flow. Although RMT was safe in acute stroke patients it had low eligibility (10% of patients screened), participation (43% of eligible patients consented) and concordance (33% of patients completed less than 50% of training).

The variability in training concordance may be interpreted as both a limitation and strength of the present study. On the one hand, from the point of view of internal validity, it is desirable that the intervention under investigation is carried out 'as prescribed'. Full concordance with the training protocol would increase confidence in the conclusion that there was lack of effect (or, conversely, confidence in the conclusion that there was a positive treatment effect, if such an effect were observed). Additional measures could have been taken in the present study to maximise participants' adherence to the intervention, such as daily visits or reminder phone calls by the investigator. On the other hand, from the point of view of external validity and from a pragmatic standpoint, concordance with active training and exercise interventions in real-life clinical practice is variable; and staff resources to supervise or prompt adherence to prescribed exercise are limited. Adherence to prescribed training as observed in the present study is likely to represent the clinical reality in NHS settings. Therefore, the findings from the present study have greater external validity and generalisability to this clinical environment than if additional measures for maximising adherence had been incorporated in the study protocol.

The rate of participant recruitment and attrition observed in this study highlights the challenge of conducting this type of research, which is set in the intense and fast-paced clinical care pathway of acute stroke; involves active engagement of participants in addition to the often already dense schedule of medical, therapy and other clinical activities; and investigates an aspect of stroke that may not intuitively seem relevant to potential participants. From a practical viewpoint, the three strategies that clearly facilitated participant recruitment and retention were: (i) the provision of transportation free of charge for participants to attend study appointments; (ii) the option to bring testing equipment to participants, to conduct measurements at their location (collaborating hospital site, participants' homes); and (iii) flexibility of the investigator to carry out study procedures during clinical 'down time', for example in the evenings or on weekends.

The intervention as applied in this study can be considered safe in acute stroke patients. It is recommended that the first training session is conducted under the supervision of an appropriately trained health care professional to adjust the appropriate level of resistance, advise on correct technique and observe for any adverse effects. The cost of delivering RMT in

the acute phase of stroke may vary. Table 35 lists relevant cost considerations. The assumption is that RMT is provided as a routine intervention to all eligible patients with the purpose of preventing pneumonia. As one worked example, one could consider the running cost (excluding overheads and staff training costs) of RMT for a patient who is provided with one device, instructed in its use for 15 minutes by a junior grade nurse, and then asked to continue training independently without further staff supervision. The estimated cost would be GBP 9.99- (unit cost) plus GBP 2.7- (staff time, based on lowest pay point of the basic Band 5 Agenda for Change pay rate, rates as of 1 April 2014) (NHS Careers, no date), totalling an estimated cost of GBP 12.69- per patient. As an alternative example, one could consider the running cost of RMT for a patient who is provided with one device, instructed in its use by a junior physiotherapist for 15 minutes, and is subsequently seen daily for 10 minutes by a healthcare assistant for supervised/assisted training over four weeks. The estimated cost would be GBP 9.99- (unit cost), plus GBP 2.7- (Band 5 physiotherapist time, pay point as above), plus GBP 45.00- (health care assistant time, based on lowest pay point of the basic Band 4 Agenda for Change pay rate, rates as of 1 April 2014), totalling an estimated cost of GBP 57.69- per patient.

Table 35. Cost considerations for delivering respiratory muscle training (RMT) in the acute phase of stroke

Cost	Considerations
Unit cost of the training device	Unit cost may vary, depending on the particular terms negotiated with the manufacturer. For the present study, the individual unit price was GBP 9.99- for both the Threshold IMT and the Threshold PEP devices, excluding freight and VAT.
Administrative costs of purchasing, storing and distributing the devices	Depending on current systems, the administration of purchase and storage of devices may create additional cost, or may be absorbed by existing systems.
Cost of staff time for delivering the intervention (one-to-one instruction and supervision)	Based on the experiences of the investigator, two possible scenarios of delivering the RMT training protocol used in this study in the acute phase of stroke could be envisaged (applicable to an inpatient setting, *i.e.* stroke unit or bedded rehabilitation unit): (a) A staff member issues the device and instructs and supervises the patient once in using the device. The patient is then encouraged to continue with training daily, but no further staff time is afforded to supervise or assist with the intervention. The estimated staff time requirement would be between 10-30 minutes, depending on the needs of the patient. (b) In addition to (a), staff members provide daily prompting and supervision with the training intervention. Staff time requirements could vary between an estimated 5-15 minutes daily in an inpatient setting, depending on patient needs. The cost of staff time will also depend on the staff grade. As a simple intervention, RMT could, for example, be administered by a junior grade nurse or physiotherapist; or an appropriately trained health care assistant or therapy assistant.
Cost of staff training	Training costs are to be considered, which includes training for all staff on a unit for familiarisation with a newly introduced intervention; and in-depth training for staff members who distribute RMT devices and instruct and supervise patients in its use. Costs will vary, depending on the number of staff members involved and staff turnover on the unit.

GBP, Pound Sterling; IMT, inspiratory muscle trainer; PEP, positive expiratory pressure; RMT, respiratory muscle training; VAT, value added tax

In this exploratory study, PECF was used as a surrogate marker for effective cough, which is an important defence against aspiration and PSP (Fontana & Lavorini 2006). Inspiratory muscle training was aimed at facilitating effective cough by increasing pre-cough lung volume, thus enabling the generation of higher expiratory cough flow. Expiratory muscle training was aimed at increasing expiratory driving pressure, resulting in higher expiratory cough flow. In previous clinical research, RMT was investigated with respect to various physiological and functional outcomes, such as respiratory muscle strength, respiratory muscle endurance, subjective perception of dyspnoea, lung function, exercise tolerance, ambulation, functional independence in daily living and health-related quality of life. In the present study, the ultimate clinical objective of RMT was to reduce PSP in the first few weeks after stroke. This exploratory study was not powered to show, and did not show, an effect on PSP incidence after stroke. A randomised controlled trial to show a 5% decrease in PSP would require 828 patients to have 80% power at the 5% significance level based on data from this study and using pair-wise comparisons (Chow *et al*. 2008) Taking into account an attrition rate of approximately 25% and a participation rate of <5%, over 22,000 acute stroke admissions would need to be screened for eligibility, which may not be feasible, even as a multicentre study. Given the lack of effectiveness of RMT to alter the physiological variables on which the assumption of PSP prevention is based, undertaking such a study may be regarded as futile.

The small sample size in the present study is a limitation, but the power of analysis was increased by applying strict inclusion criteria, increasing the sample size following interim analysis of the observed variations in the primary outcome and undertaking pooled analysis. Variable training concordance is a potential source of bias, but neither comparison based on training intensity nor sensitivity analysis showed significant differences in outcomes. Correlation analysis of training concordance with training effects showed weak correlations. Although the p-values were largely non-significant, this suggests that the number of training breaths did not considerably influence the change in outcomes observed. This lends further support to the overall interpretation of the findings that the investigated training intervention did not show any effect in improving these outcomes. Missing data, confounding due to non-significant differences in baseline prognostic variables and multiple testing are sources of potential

statistical bias, but these were minimised by using regression with predictive model-based multiple imputation techniques for analysis and correcting for multiple testing.

5.6 Conclusion

With respect to the study aim, it can be concluded that RMT delivered at the frequency and intensity as applied in this study was not effective in increasing peak expiratory flow of voluntary and reflex cough or expiratory and inspiratory maximal mouth pressures when compared with sham respiratory muscle training. Training concordance varied widely and is acknowledged as a limitation to the study. However, it is suggested that this reflects adherence to prescribed exercise in real life clinical practice, thereby giving the study greater external validity than if additional measures for maximising concordance had been incorporated.

RMT delivered at the frequency and intensity as applied in this study can be considered a safe and generally well tolerated intervention in acute stroke patients who do not have raised intra-cranial pressure or acute cardiac conditions. RMT did not affect participants' vital parameters, except in one case where the participant became hypertensive and experienced subjective discomfort after training. Several participants experienced light-headedness during or after RMT, which was related to hyperventilation and resolved with increasing number of rests between breaths. It is therefore recommended that the first session of RMT is supervised by an appropriately trained health professional to observe for potential adverse effects and to adjust the training procedure to avoid light-headedness.

Due to the lack of demonstrable intervention effect, it was not possible to identify a specific subpopulation of stroke patients who are likely to gain from the intervention as delivered in this study. For researchers who are interested in continuing this type of research, we suggest that the target population should be those acute stroke patients who (i) are identified as being at risk of aspiration (*i.e.* have an unsafe swallow); and (ii) have low voluntary cough flow values,

predisposing them to an increased risk of pneumonia and giving adequate scope for physiological improvement in cough flow (From the data observed, we would recommend an estimated upper threshold of approximately 500 to 600 L/min). Narrowing the inclusion criteria accordingly would impact on the number of eligible participants and therefore the feasibility and timescales of any such research project.

RMT, as delivered in this study, was found to be a relatively safe and low-cost intervention, and therefore, from a practicality standpoint, its implementation within the NHS stroke care pathway would be feasible. At this time, based on the evidence available from this and other studies, RMT is not considered a relevant treatment intervention in the acute care of stroke patients. Further research of RMT in stroke might be warranted.

To conclude, this study shows that although RMT is safe in acute stroke patients, only a small number of incident stroke patients are eligible, will participate and be concordant with respiratory muscle training protocols. As these have no significant effect on increasing muscle strength or cough effectiveness, RMT is unlikely to make a clinically meaningful difference in reducing PSP in acute stroke patients. Hence, further trials of RMT with the clinical endpoint of PSP prevention may not be warranted.

Chapter 6 Relationship between cough flow and incidence of post-stroke pneumonia according to swallow safety

6.1 Introduction

The association between impaired swallow and increased risk of pneumonia in stroke patients is well established (Ickenstein *et al*. 2010, Lakshminarayan *et al*. 2010, Hinchey *et al*. 2005, Martino *et al*. 2005), and studies show a five- to eleven-fold increase in risk of PSP with dysphagia and aspiration (Martino *et al*. 2005).

The occurrence of PSP depends not only on aspiration but also on the integrity of mechanical and immunological defense mechanisms (Hannawi *et al*. 2013; Teramoto 2009). Of these, cough is the most immediate and important defense against the development of PSP in stroke patients with aspiration (Widdicombe *et al*. 2011). Studies show that voluntary and reflex cough are both significantly impaired in acute stroke patients (Zhou *et al*. 2012, Yoon *et al*. 2011, Ward *et al*. 2010, Harraf *et al*. 2008). Reduced or absent reflex cough sensitivity has been shown to be associated with higher incidence of PSP (Masiero 2008, Addington *et al*. 2005, 1999a, Holas 1994). Expiratory cough flow, however, has not been examined as a risk factor for PSP in acute stroke. Figure 18 gives a schematic of the current available evidence on the interactions between swallow, pneumonia and cough in stroke, including studies demonstrating an association between degree of swallowing impairment and reduction in cough flow (Kimura *et al*. 2013, Smith Hammond *et al*. 2009, 2001).

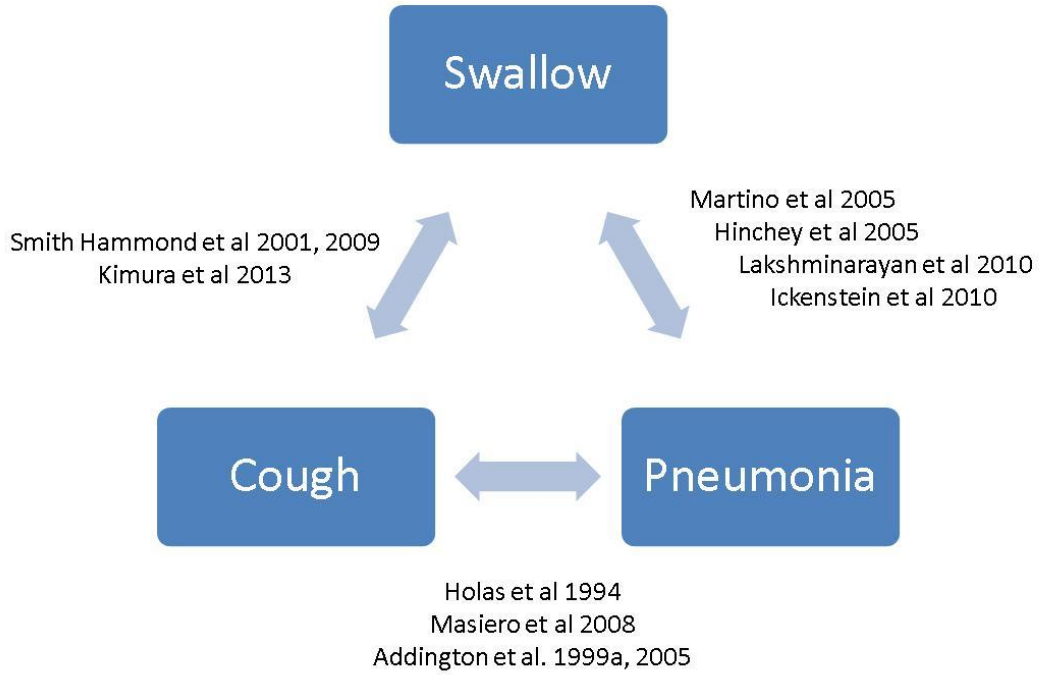


Figure 18. Inter-relationships between swallow, cough and pneumonia and studies examining the respective two-way interactions in stroke populations.

The aim of the present study was to explore the relevance of PECF as a predictor/mediator in the relationship between swallowing impairment and pneumonia in acute stroke. The hypothesis was that higher PECF would be protective against the risk of PSP in acute stroke patients with swallowing problems. The specific objectives were:

- To investigate the association between PECF of voluntary and induced reflex cough, and the development of PSP in stroke patients stratified by swallowing status.
- To quantify the interaction between PECF, aspiration risk and pneumonia risk.

6.2 Methods

6.2.1 Design

This was an exploratory study of PECF as a predictor variable for pneumonia incidence in stroke. A secondary analysis was conducted, using data from a randomised controlled trial of RMT in stroke (study reference ISRCTN40298220) (chapter 5). For the purpose of this study, the data structure assumed was that of a prospective longitudinal observational study for identification of a diagnostic predictor.

6.2.2 Setting

Participants were recruited at King's College Hospital, a comprehensive stroke centre in London, UK. Participants could be discharged home or transferred to stroke rehabilitation units in local hospitals during the study period. Patients received standardised stroke rehabilitation on accredited units or from supported discharge teams at home.

6.2.3 Participants

Acute haemorrhagic or ischemic stroke patients aged 18 years and above were recruited within two weeks of stroke onset. Inclusion criteria were: NIHSS score of 5-25 with motor impairment; and ability to give informed consent and follow study procedures. Exclusion criteria were: poorly controlled hypertension (blood pressure >180/100 on three or more occasions in 24 hours); myocardial infarction, angina or acute heart failure in the preceding three months; pulmonary disease including asthma and COPD; neurological conditions other than stroke; and orthopaedic conditions adversely affecting the respiratory pump. Written informed consent was obtained prior to inclusion and the study was approved by the UK NRES (Wandsworth Research Ethics Committee, study reference 10/H0803/32).

6.2.4 Baseline assessments

Baseline assessments were conducted within 2 weeks of stroke onset and included patient demographics, stroke characteristics and respiratory parameters. Swallowing function was described according to swallow screens and clinical bedside assessments, which were conducted as part of routine acute stroke care (Appendix 2). Respiratory assessments at baseline were forced spirometry, maximal mouth pressure measurements and cough flow measurements of volitional and capsaicin-induced reflex cough. A detailed description of respiratory assessment methods is given in chapter 3.

6.2.5 Outcome assessment

The outcome of interest was incidence of pneumonia, which was observed for four weeks following baseline assessment and determined from medically documented diagnosis of pneumonia or prescription of antibiotics for pneumonia.

6.2.6 Sample size

The study was a secondary analysis of available clinical trial data. No prospective sample size calculation was conducted with respect to the statistical analyses presented here.

6.2.7 Statistical analysis

The statistical analysis approach was hypothesis-driven, examining only the predictor PECF of voluntary and reflex cough according to aspiration risk. First, the sample was stratified according to aspiration risk, into those with safe swallow (low risk of aspiration) and those with unsafe swallow (high risk of aspiration). Predictor (PECF) and outcome variables (pneumonia

incidence) between the two groups were compared using the appropriate test for comparison of independent samples.

Second, statistical methods for diagnostic accuracy (receiver-operated characteristics (ROC) curve, sensitivity, specificity, positive predictive value, negative predictive value, false-positive rate, false-negative rate, accuracy, likelihood ratio, and pre- and post-test odds) were applied to assess predictor characteristics of PECF with respect to outcome PSP (Altman 1991, pp 409-419). Statistics for diagnostic accuracy were calculated from logistic regression models, where the independent variable was expressed as an interaction between swallow safety and PECF, *i.e.* the model included two different models of association between cough flow and pneumonia, depending on swallow safety (as opposed to confounding, where one single model is generated for the association between two variables in the presence of a confounder variable). Model goodness of fit was assessed using Pearson chi-squared and Hosmer-Lemeshow tests (StataCorp 2013, pp. 958-961).

Third, exact logistic regression (StataCorp 2013, pp. 507-528, Mehta & Patel 1995) was conducted to quantify how baseline PECF in interaction with swallow safety modifies four-week risk of PSP. Exact logistic regression is an alternative to the standard maximum likelihood based logistic regression estimator, and yields greater statistical precision in small samples and in scenarios where outcomes are rare (StataCorp 2013, pp. 507-508). All data analyses were conducted using Stata statistical software (Stata v12.1, StataCorp, College Station, Texas).

6.3 Results

6.3.1 Baseline characteristics

The sample consisted of 72 participants. Baseline characteristics are given in Table 36. When the sample was stratified by aspiration risk, patients with unsafe swallow (n=33) were older

(mean (SD) age 70.2 vs 59.9 years, p=0.0022) and had more severe stroke impairment (median (IQR) NIHSS score 9 (7, 14) vs 6 (5, 10), p=0.0002) than patients with safe swallow. Patients with unsafe swallow also performed worse on a number of variables of forced spirometry (FVC, FEV_1, PEF), voluntary cough (PECF, CVI, CVE, CVAC), reflex cough (PICF, CVI), and respiratory muscle strength (PEmax, PImax) (Table 36).

Table 36. Baseline characteristics of the sample

		Stratification by aspiration risk		
	Total sample (n=72)	Safe swallow (n=39)	Unsafe swallow (n=33)	p-value
Age (years)	64.6 (14.4)	59.9 (14.0)	70.2 (13.1)	0.0022
Males	42 (58%)	23 (59%)	19 (59%)	0.905
NIHSS score (median, IQR)	8 (5, 12)	6 (5, 10)	9 (7, 14)	0.0002
Pre-morbid NEADL score (median, IQR)	60 (46, 63)	60 (54, 63)	57 (35, 63)	0.203
Stroke Type				
Ischemic	65 (90%)	38 (97%)	27 (82%)	0.089
Haemorrhagic	7 (10%)	1 (3%)	6 (18%)	0.089

Table 36. continued

Stroke Side				
Left	26 (36%)	16 (41%)	10 (30%)	0.393
Right	45 (62%)	22 (56%)	23 (70%)	0.393
Bilateral	1 (1%)	1 (3%)	-	0.393
Stroke Site				
Cortical	33 (46%)	17 (44%)	16 (48%)	0.578
Subcortical	31 (43%)	19 (49%)	12 (36%)	0.578
Brainstem/cerebellar	8 (11%)	3 (8%)	5 (15%)	0.578
Current smoker	18 (25%)	10 (26%)	8 (24%)	0.891

Table 36. continued

Forced spirometry				
FVC (L)	2.2 (1.0)	2.6 (0.9)	1.8 (1.0)	0.0008
FEV_1 (L)	1.8 (0.8)	2.0 (0.8)	1.5 (0.8)	0.0071
PEF (L/min)	240 (138)	274 (146)	199 (118)	0.0070
Maximal voluntary cough				
PICF (L/min)	134 (73)	146 (80)	119 (61)	0.109
PECF (L/min)	465 (258)	535 (262)	383 (230)	0.011
CVI (L)	1.6 (0.8)	1.8 (0.7)	1.3 (0.8)	0.011
CVE (L)	1.3 (0.7)	1.5 (0.7)	1.1 (0.7)	0.042
CVAC (L/s/s)	166 (113)	194 (119)	134 (99)	0.024
GCT (s)	0.24 (0.2)	0.26 (0.2)	0.21 (0.1)	O.223

Table 36. continued

Capsaicin-induced involuntary cough				
PICF (L/min)	88 (44)	98 (51)	77 (32)	0.046
PECF (L/min)	283 (114)	303 (110)	260 (116)	0.126
CVI (L)	1.2 (0.6)	1.3 (0.7)	1.0 (0.5)	0.024
CVE (L)	0.7 (0.4)	0.7 (0.4)	0.6 (0.3)	0.406
CVAC (L/s/s)	114 (50)	124 (49)	102 (50)	0.073
GCT (s)	0.20 (0.1)	0.19 (0.1)	0.22 (0.1)	0.345
Maximal mouth pressures				
PEmax (cmH_2O)	59 (34)	71 (35)	40.5 (25)	0.0005
PImax (cmH_2O)	43 (29)	53 (30)	31 (23)	0.0013

Figures are mean (SD) and frequency (%), unless stated otherwise

p-values are for between-group comparison of patients with safe and unsafe swallow and were calculated using the appropriate parametric (independent samples t-

test) or non-parametric (Mann-Whitney U test) statistical significance test for continuous data, and Chi squared or Fisher's exact test for categorical data (5% significance level alpha, 80% power)

CVAC, cough volume acceleration; CVE, cough volume expired; CVI, cough volume inspired; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; GCT, glottis compression time; NEADL, Nottingham Extended Activities of Daily Living questionnaire; PECF, peak expiratory cough flow; PEF, peak expiratory flow; PEmax, maximal expiratory mouth pressure; PICF, peak inspiratory cough flow; PImax, maximal inspiratory mouth pressure

6.3.2 Predictor and outcome variables according to aspiration risk

Out of 72 participants, 13 (18%) developed pneumonia within four weeks from baseline assessment. In the safe swallow group, two (5%) out of 39 patients developed pneumonia. There was no statistically significant difference between PECF of either voluntary or involuntary cough in the safe swallow group between patients who developed pneumonia and those who did not (Table 37). In the unsafe swallow group, eleven (33%) out of 33 patients developed pneumonia. In patients who developed pneumonia, mean PECF of voluntary cough was lower by 196 L/min (p=0.0053). Mean PECF of involuntary cough was also lower by 45 L/min in patients who developed pneumonia, although this was not statistically significant (p=0.277) (Table 37).

Table 37. Peak expiratory cough flow (PECF) according to four-week incidence of pneumonia in patients with low aspiration risk (safe swallow) and high aspiration risk (unsafe swallow)

	Low risk of aspiration (safe swallow)			High risk of aspiration (unsafe swallow)		
	No pneumonia (n=37)	Pneumonia (n=2)	p-value	No pneumonia (n=22)	Pneumonia (n=11)	p-value
Voluntary cough PECF (L/min)	535 (264)	546 (307)	0.917	448 (244)	252 (130)	0.0053
Capsaicin-induced involuntary cough PECF (L/min)	301 (110)	324 (168)	0.945	276 (124)	231 (100)	0.277

Figures are mean (SD)

p-values were calculated using independent samples t-test with unequal variance

6.3.3 Diagnostic accuracy of peak cough flow in interaction with aspiration risk for outcome pneumonia

The ability to predict the outcome pneumonia with a probability of >0.5 from the interaction between PECF and aspiration risk was modelled for both voluntary and reflex cough. Appendix 4 gives full details of Stata commands and outputs. Model goodness-of-fit tests were satisfactory, with p-values >0.05.

For voluntary cough PECF, the model yielded 46.15% sensitivity and 93.22% specificity. Positive and negative predictive values were 60.0% and 88.71%, respectively. The false positive rate was 6.78% in all patients who did not develop the outcome; and 40% in all patients for whom pneumonia had been predicted. The false negative rate was 53.85% in all patients who did develop pneumonia; and 11.29% in all patients who had been predicted not to develop pneumonia. The overall accuracy (all cases correctly classified) was 84.72%. The area under the ROC curve was 0.836 (Figure 19). Sensitivity and specificity according to probability cut-off are shown in Figure 20.

The positive likelihood ratio for this model was 6.8, and the negative likelihood ratio was 0.58. Assuming a PSP incidence over four weeks of 15%, the pre-test odds of developing PSP are 0.18 (two to eleven, in favour of not developing pneumonia). A positive model prediction therefore resulted in post-test odds of 1.22 for developing PSP (approximately ten to eight, in favour of developing pneumonia); and a negative model prediction almost halved the odds of developing pneumonia to 0.10 (one to ten, in favour of not developing pneumonia).

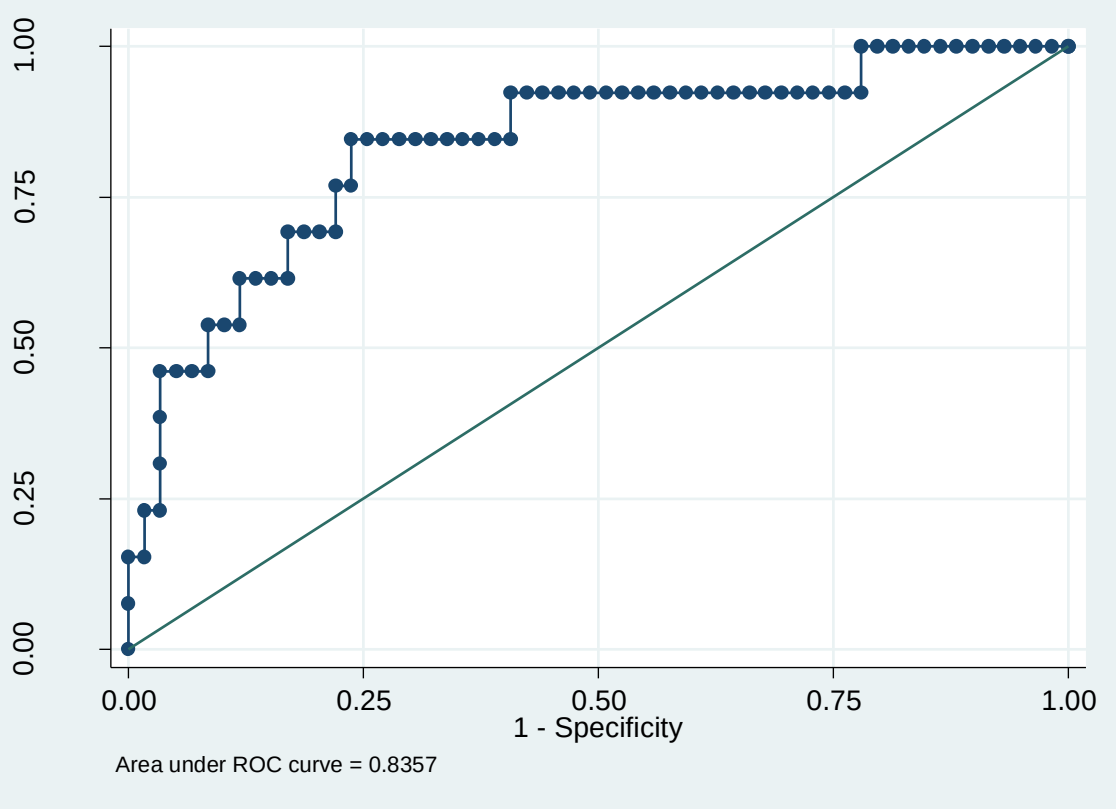


Figure 19. Receiver operating characteristic (ROC) curve for logistic regression model predicting outcome pneumonia from interaction between voluntary peak cough flow and aspiration risk

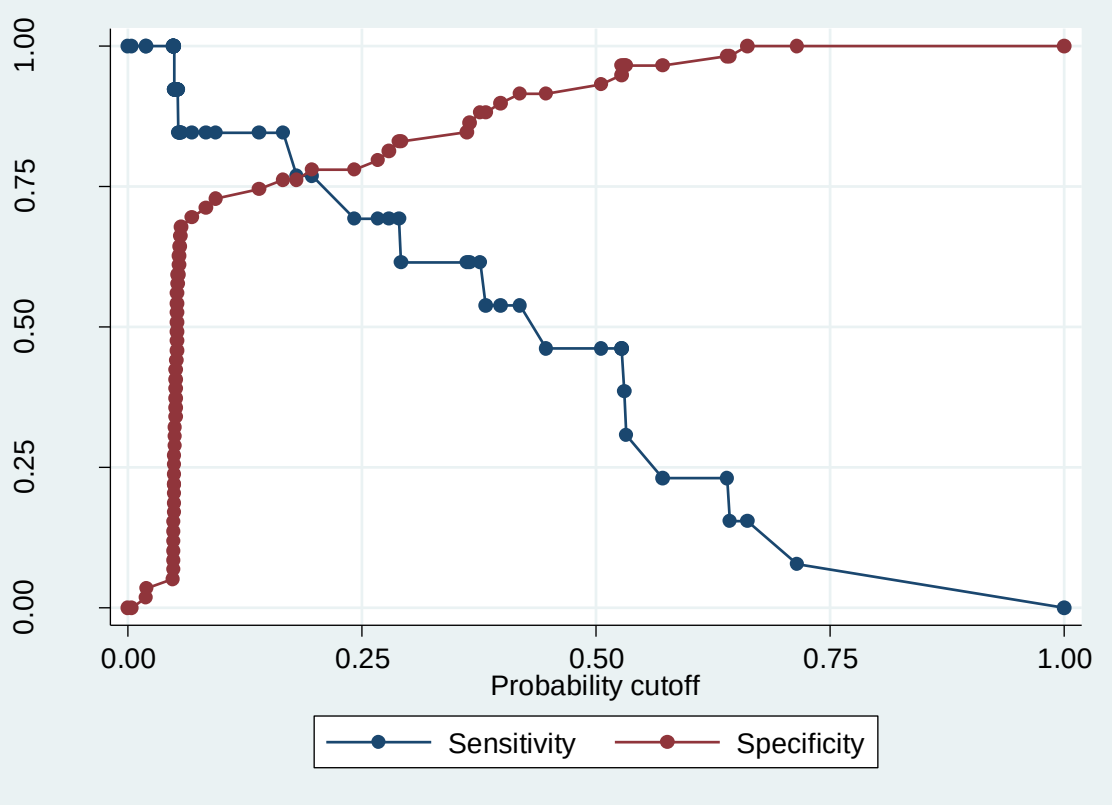


Figure 20. Sensitivity and specificity according to probability cut-off for logistic regression model predicting outcome pneumonia from interaction between voluntary peak cough flow and aspiration risk

For capsaicin-induced involuntary cough PECF, the model failed to predict any of the pneumonia cases; although the model correctly identified 82.61% of cases who did not develop pneumonia, and the area under the ROC curve was 0.768.

A third model was explored, adjusting the interaction between voluntary cough PECF and aspiration risk with the confounders age, sex and stroke severity. In addition to swallowing impairment, these three risk factors for PSP are the most frequently identified in the literature. This model also showed satisfactory goodness-of-fit and yielded 53.85% sensitivity and 93.22% specificity. Positive and negative predictive values were 63.64% and 90.16%, respectively. The false positive rate was 6.78% in all patients who did not develop the outcome; and 36.36% in all

patients for whom pneumonia had been predicted. The false negative rate was 46.15% in all patients who did develop pneumonia; and 9.84% in all patients who had been predicted not to develop pneumonia. The overall accuracy (all cases correctly classified) was 86.11%. The area under the ROC curve was 0.855 (Figure 21). Sensitivity and specificity according to probability cut-off are shown in Figure 22.

The positive likelihood ratio for this model was 7.9, and the negative likelihood ratio was 0.50. Based on a PSP incidence of 15% and pre-test odds of developing PSP of 0.18 (two to eleven, in favour of not developing pneumonia), a positive model prediction therefore resulted in post-test odds of 1.42 for developing PSP (approximately ten to seven, in favour of developing pneumonia); and a negative model prediction reduced the odds of developing pneumonia to 0.09 (one to eleven, in favour of not developing pneumonia).

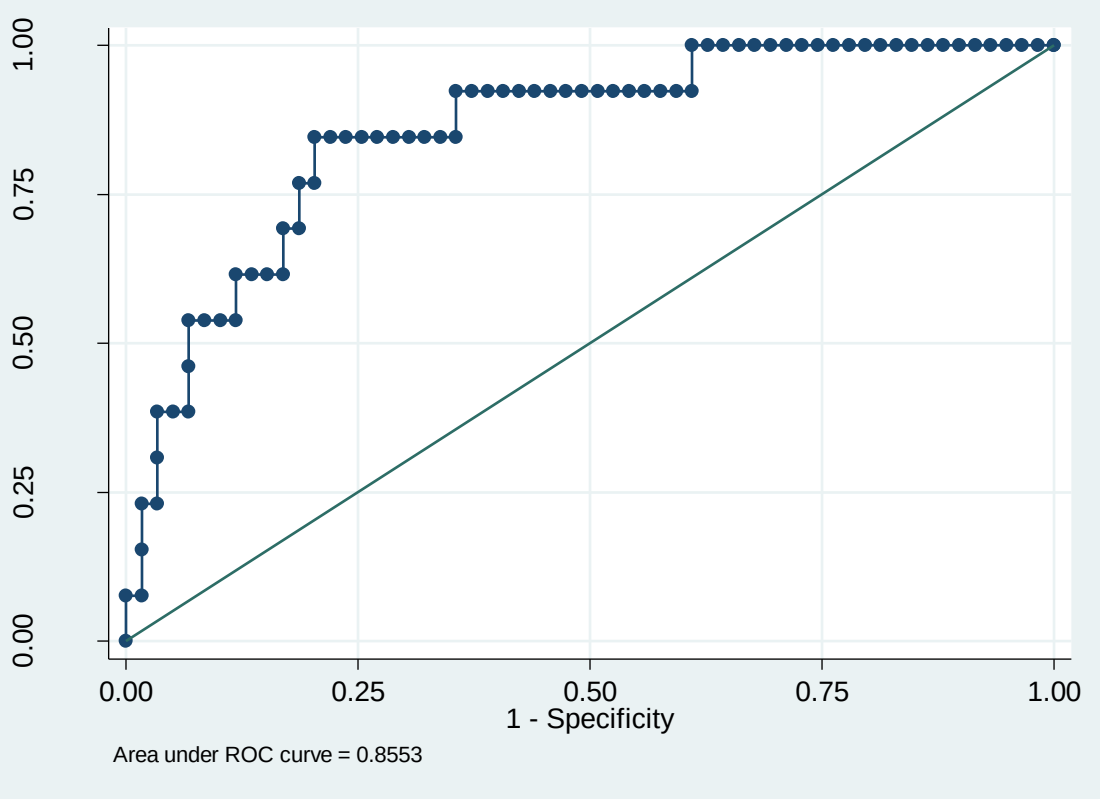


Figure 21. Receiver operating characteristic (ROC) curve for logistic regression model predicting outcome pneumonia from interaction between voluntary peak cough flow and aspiration risk, and adjusted for age, sex and stroke severity

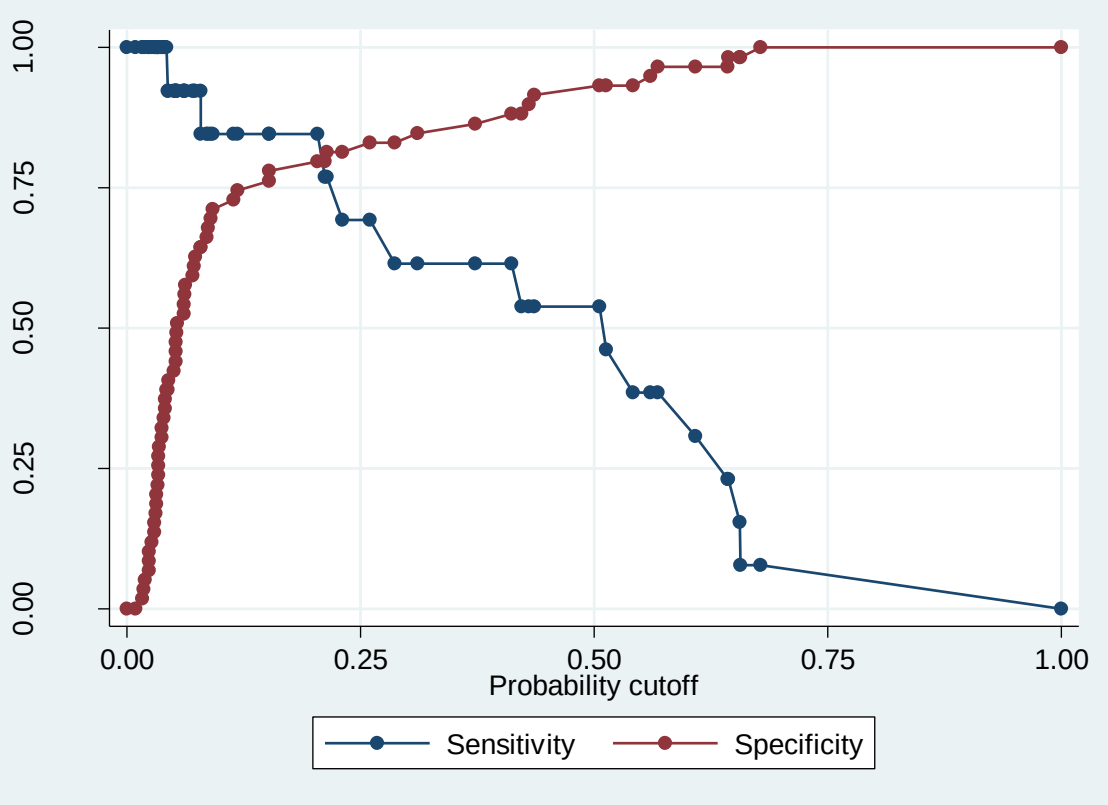


Figure 22. Sensitivity and specificity according to probability cut-off for logistic regression model predicting outcome pneumonia from interaction between voluntary peak cough flow and aspiration risk, and adjusted for age, sex and stroke severity

6.3.4 Modification of pneumonia risk according to cough flow

Exact logistic regression was used to model how PSP risk changes according to magnitude of cough flow. To fit the model, the continuous variable peak cough flow had to be categorised. Increments of 50 L/min were selected for categorisation, which lies just above the average test-retest variability to be expected in the absence of change (chapter 3). Stata commands and outputs for the applied exact logistic regression models are given in Appendix 5.

Analysis showed that for patients at high risk of aspiration (unsafe swallow), each increase in voluntary cough PECF by 50 L/Min was associated with a statistically significant decrease in

pneumonia risk (OR 0.73, 95%CI 0.51-0.95, p=0.012). For involuntary cough PECF, each increase by 50 L/min was also associated with a reduction in pneumonia risk, although this was not statistically significant (OR 0.87, 95%CI 0.60-1.20, p=0.45). For patients at low risk of aspiration (safe swallow), there was no association between PECF of either voluntary (OR 1.01, 95%CI 0.74-1.33, p=0.89) or involuntary cough (OR 1.11, 95%CI 0.55-2.23, p=0.84) and pneumonia. In Figures 23 and 24 the probability of developing pneumonia is plotted according to PECF. For patients who were at high risk of aspiration and who had voluntary cough flow of >600 L/min, the probability of pneumonia was equivalent to patients who were at low risk of aspiration. The shapes of the 95% confidence intervals in Figures 23 and 24 are explained by the distribution of data points across the PECF range, which was denser where confidence intervals are narrower, and less dense where confidence intervals widen.

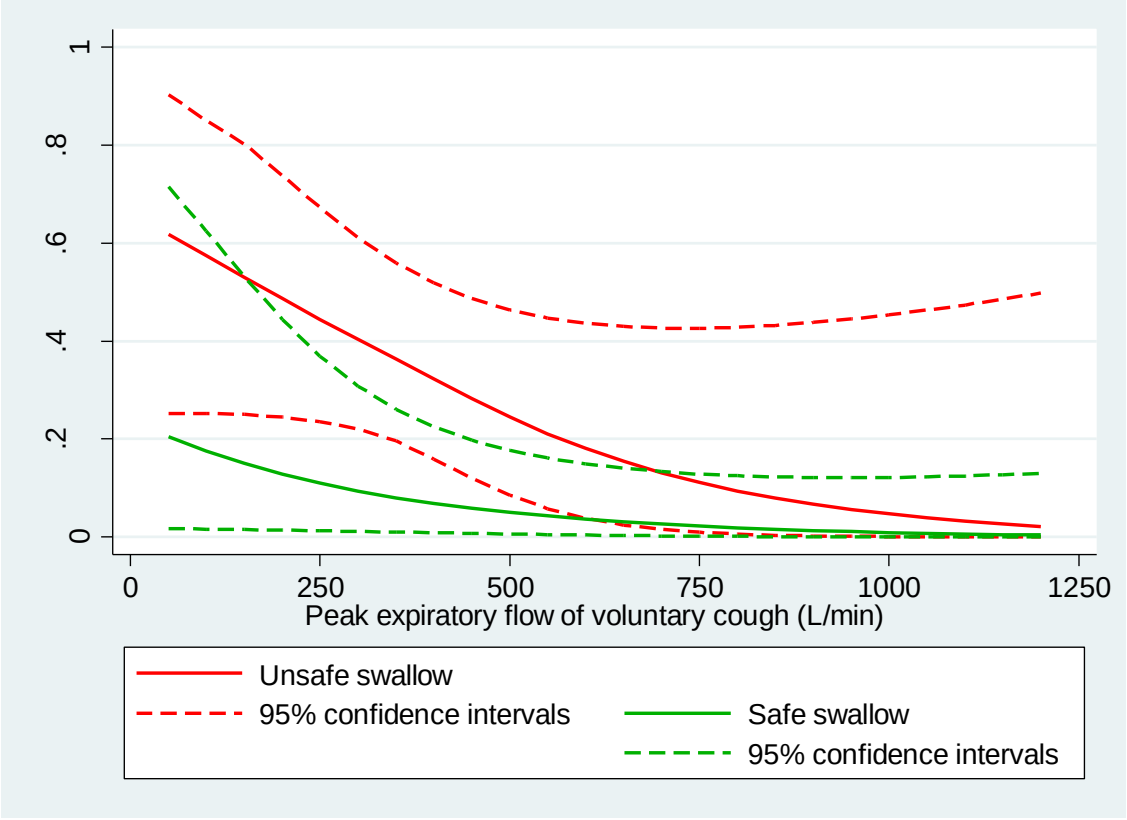


Figure 23. Probability of pneumonia, according to swallow safety and peak expiratory flow of voluntary cough at baseline

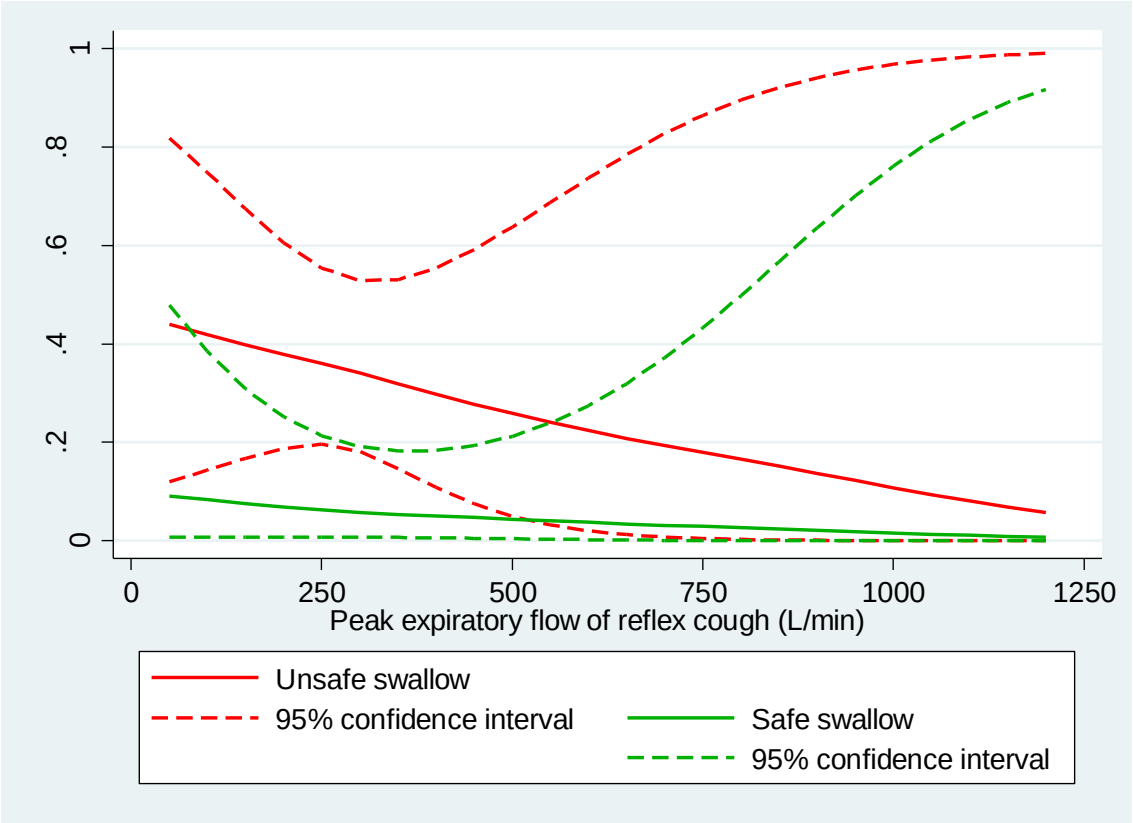


Figure 24. Probability of pneumonia, according to swallow safety and peak expiratory flow of capsaicin-induced involuntary cough at baseline

6.4 Discussion

This exploratory study demonstrated that PECF of voluntary cough, measured within two weeks of stroke onset, was associated with the four-week risk of developing PSP in patients with high aspiration risk. It was shown that the interaction between aspiration risk and voluntary cough PECF can be used to refine the odds of developing PSP. The model performed stronger in predicting negative outcome (no development of PSP) than positive outcome. The association between the magnitude of voluntary cough PECF and the probability of outcome PSP was also demonstrated. PECF had minimal influence on PSP probability in patients with safe swallow; however, in patients with unsafe swallow, the model showed decreasing PSP probability with

increasing peak cough flow. From a PECF of approximately 600 L/min upwards, PSP probability was reduced to that equivalent in patients with safe swallow. Overall, these data support the hypothesis and logic model that stronger cough, as measured by expiratory cough flow, is protective of PSP in patients who are at risk of aspiration.

A number of limitations to this study are acknowledged. First, this was an exploratory, secondary analysis of clinical trial data. Investigations of diagnostic predictors are generally addressed in observational study designs. While these data were collected prospectively, which can be regarded as a strength in comparison with a retrospective observational design, the study sample is selective due to the trial eligibility criteria. The incidence of PSP (18%) within this sample is in the higher range of reported pneumonia incidence rates in non-ICU settings (median (IQR) 10% (6.4, 16.2) (Hannawi *et al*. 2013). This influences the performance of the predictor models, which depends on the baseline incidence. Post-estimation odds according to varying baseline incidence are given in Table 38. These post-estimation odds demonstrate that the predictor models are more informative in identifying patients who are unlikely to develop PSP (halving the pre-estimation odds), but less informative in predicting who will develop PSP.

Table 38. Post-estimation odds for developing post-stroke pneumonia (PSP) as predicted from aspiration risk and voluntary cough PECF, and according to varying PSP incidence

		Model variables			
		Voluntary cough PECF and aspiration risk		Voluntary cough PECF, aspiration risk, age, sex and stroke severity	
Incidence of PSP	Pre-test odds of developing PSP	Odds of developing PSP	Odds of not developing PSP	Odds of developing PSP	Odds of not developing PSP
5%	0.05	0.34	0.029	0.40	0.025
10%	0.11	0.75	0.064	0.87	0.055
15%	0.18	1.22	0.10	1.42	0.09
20%	0.25	1.50	0.14	1.98	0.12
25%	0.33	2.24	0.19	2.61	0.16
30%	0.43	2.92	0.25	3.40	0.22

Second, the use of trial data for this secondary analysis was considered appropriate, since the trial intervention (respiratory muscle strengthening to improve PECF) did not show an effect. It is possible, however, that participation in the experimental and sham groups introduced unknown bias. Third, the study sample was small, and the outcome PSP occurred in only two patients in the safe swallow group. Statistical precision was maximised by examining only one association of interest, which was defined *a priori*; and by using exact logistic regression. Commonly, models are developed using stepwise regression approaches to statistically identify the most relevant predictors from a number of potential candidate variables. The validity of these approaches depends on the sample size, which needs to be larger the more candidate

variables are added to the analysis. From the baseline variables of the present sample, other potential predictor variables for PSP may be considered relevant, *e.g.* FVC, FEV_1, PEF, PEmax and PImax. These respiratory parameters all link with cough function and all showed statistically significant differences when the sample was stratified according to swallow safety. However, the small sample precluded a valid exploration of these parameters in comparison with PECF, and PECF was selected *a priori* as the most immediate parameter of cough effectiveness.

Despite these limitations, the present analysis provides valuable information in a little researched field. The importance of cough is frequently discussed in the context of swallowing impairment and aspiration pneumonia. However, to our knowledge there is no published evidence demonstrating the association between cough intensity and pneumonia risk after stroke. The notion that strong cough protects from aspiration pneumonia is a generally held clinical belief, probably because of its intuitive plausibility. Some evidence is available from patients with degenerative neuromuscular conditions, which shows that weaker cough is associated with increased risk of failed weaning from mechanical ventilation and with increased risk of hospitalisation due to pulmonary complications (Bach *et al*. 1997, Bach & Saporito 1996). Cough intensity is not routinely measured in clinical practice and may therefore not be conveniently studied as a risk factor for PSP. The data presented here therefore provided an opportunity to explore the role of cough intensity in interaction with swallowing function and pneumonia risk in the acute phase of stroke, and the hypothesis and logic model were indeed supported by the data. In view of the study limitations, it would be desirable to replicate the findings in an observational study design, with a larger sample that is representative of the majority of patients with incident stroke.

Overall, these data demonstrate that measurements of peak expiratory flow during cough could find clinical utility in quantifying PSP risk, in particular for categorising patients to high or low risk, according to a certain PECF threshold. With respect to the absolute value of such a PECF threshold, the potential for considerable inaccuracy between different flow measurement devices has to be taken into account (chapter 3). The threshold value of 600 L/min suggested

by the present study therefore needs to be used with reference to the particular measurement system applied in this study.

6.5 Conclusion

These data show that the risk of PSP decreases in proportion with increasing PECF of voluntary cough in stroke patients who are at risk of aspiration, but not in patients with safe swallow. This association is more pronounced and statistically significant for voluntary PECF, compared with PECF of involuntary cough. These data confirm that stronger cough protects from aspiration-related pneumonia after stroke. A threshold value for voluntary cough PECF may be useful in clinical practice to categorise patients according to PSP risk; however, routine clinical application is dependent on the availability of portable devices that can measure peak cough flow accurately in clinical settings.

Chapter 7 Longitudinal observation of cough frequency in the first three months following acute stroke

7.1 Introduction

Cough frequency is a parameter of interest in cough research, in particular in studies of excessive coughing and cough suppressants, or where cough is examined as a positive clinical sign of respiratory disease (Sunger *et al*. 2013, Decalmer *et al*. 2012, Birring 2011, Morice *et al*. 2007, Raj & Birring 2007, Birring *et al*. 2006). Few studies have examined cough frequency in the context of impaired swallowing and pneumonia risk, an interaction that is relevant in patients with neurogenic swallowing dysfunction. As one example, the occurrence of coughing bouts and choking episodes was investigated in one study of patients with motor neuron disease (Hadjikoutis *et al*. 2000). Using a self-report diary over a three day period, it was found that 27 out of 37 patients with motor neuron disease experienced between one and 50 coughing and choking episodes, while ten patients did not report any coughing or choking. This was compared with a group of 23 healthy control subjects, out of whom two subjects reported one to two episodes of coughing or choking and 21 reported none. Coughing and choking in patients with motor neuron disease coincided partly with meal times, but occurred equally often outside meal times. Higher frequency of coughing and choking was associated with poorer performance in volitional respiratory and cough function tests (forced spirometry, peak flow and sound level during maximal volitional cough). Out of 37 patients, only two had developed a chest infection since disease onset, and both these patients had reported coughing and choking episodes. None of the ten patients who did not report any coughing or choking episodes had developed chest infections since the onset of the condition.

As in the study by Hadjikoutis and colleagues, cough frequency assessments can complement investigations of swallowing function and pneumonia risk in neurological patient groups. Aspiration of food and drink during the act of swallowing may trigger protective coughs. Increased cough frequency, for example during mealtimes, may therefore be interpreted as a

warning sign that the person is dysphagic and at increased risk of aspiration and aspiration pneumonia (Miller & Britton 2011, pp. 23-42, Singh & Hamdy 2006, Ramsey *et al*. 2003). Relating this scenario to the acute stroke population, it may be hypothesised that increased cough frequency will correlate with presence of dysphagia, risk of aspiration and risk of pneumonia.

An alternative hypothesis may postulate that reduced cough frequency will identify lack of reflexive protection from aspiration in dysphagic patients, therefore indicating greater risk of aspiration and aspiration pneumonia. Other than in motor neuron disease, where the sensory afferents are generally not affected by the disease process, stroke can lead to an impairment of the sensory afferents and central nervous pathways processing cough as well as the efferent pathways effecting cough. The potential for stroke to reduce reflex cough sensitivity has been demonstrated (Addington *et al*. 2005, 1999a). As a result, cough frequency may be diminished in a portion of acute stroke patients.

To date, cough frequency has not been described in acute stroke. Longitudinal observation of cough frequency is of interest, to allow comparison with normative values and describe trends of recovery.

7.2 Aims and objectives

The aim of the present study was to explore cough frequency in a cohort of acute stroke survivors. Specific study objectives were:

- To validate the Leicester Cough Monitor (LCM) as a method of cough frequency measurement in acute stroke
- To describe 24-hour cough frequency longitudinally over twelve weeks
- To compare the cough frequency in stroke survivors with normative values

- To explore relationships between cough frequency and other parameters, *e.g.* swallowing impairment, lung function and cough intensity

7.3 Methods

7.3.1 Participants

Acute haemorrhagic or ischemic stroke patients aged 18 years and above were recruited within two weeks of stroke onset. Patients were recruited consecutively from the hyper-acute stroke unit at one tertiary stroke centre in London, UK. Inclusion criteria were: NIHSS score of 5-25 with motor impairment; and ability to give informed consent and follow study procedures. Exclusion criteria were: poorly controlled hypertension (blood pressure >180/100 on three or more occasions in 24 hours); myocardial infarction, angina or acute heart failure in the preceding three months; pulmonary disease including asthma and COPD; neurological conditions other than stroke; and orthopaedic conditions adversely affecting the respiratory pump. Written informed consent was obtained prior to inclusion and the study was approved by the UK NRES (Wandsworth Research Ethics Committee, study reference 10/H0803/32).

7.3.2 Cough frequency measurements

Cough frequency measurements were made using the LCM, a semi-automated cough frequency measurement system (Birring *et al*. 2008, Matos *et al*. 2007, Matos *et al*. 2006). This device consists of a portable sound recording device (Digital Voice Tracer LFH0662, Philips Electronics UK Ltd, Guildford, England), which is worn in a pouch or pocket. A small microphone is clipped onto the patient's collar or lapel, as close as possible to the anterior neck. A continuous sound recording is made, generally over an entire 24-hour period. The digital recording is then processed through accompanying computer software (Leicester Cough Monitor, Version 2.0, October 2012, lcmonitor@gmail.com), which automatically registers sound

patterns typical for cough. During data processing, a human operator listens back to examples of identified sound patterns and confirms whether or not the identified sound is in fact a cough sound. Sounds sometimes misidentified by the software as cough sounds are, for example, short sharp sounds such as the closing of doors or dropping of objects. The automated cough count is refined through the feedback provided by the human operator. The software output lists the total number of cough events over the entire time period of recording; the average number of cough events per hour; the total number of cough bouts; the average number of cough bouts per hour; the average number of cough events per bout; and the day time and night time values for each of these statistics.

7.3.3 Study design

7.3.3.1 Validation of the Leicester Cough Monitor in acute stroke

To validate the LCM for acute stroke patients, a validation study was conducted with a group of five participants. Participants were inpatients on the acute stroke unit at the time and shared an open hospital bay with up to three other patients. Potential concerns regarding the validity of the sound-based LCM system in this environment were that coughs from patients sharing the room, members of staff or visitors could contaminate the patient cough count and result in higher measurements; or that for patients with severely weakened or atypical sounding cough (*e.g.* 'bovine' cough sound in bulbar involvement) the LCM software would not recognise coughs, resulting in lower measurement. Participants wore the LCM for 15 minutes, while a researcher observed the participant from close proximity 'live' for the entire duration of the recording. The researcher conducted a 'live' count of coughs by the participant (patient coughs), and of all other coughs which could be heard by the researcher, including coughs from other patients, staff and visitors within the area (ambient coughs). To ensure that there were cough events to be recorded, the participant and other people in the room were prompted to cough several times during the 15 minutes recording.

LCM recordings were processed using the accompanying computer software. When giving operator input to cough sound recognition, the researcher consciously assessed for each sound sample presented by the programme: (a) whether the sound was a cough sound (designating sounds that did not resemble cough sounds as 'non-cough'); (b) if the sound was a cough sound, whether it sounded distant or near on the recording (designating distant sounding coughs as 'non cough'); (c) if the sound was a cough sound, whether it sounded as if it generated from a person of the opposite sex to the test subject (designating coughs from persons of the opposite sex as 'non-cough'); and (d) whether the test subject had a particularly characteristic sounding cough, such as a bovine or wheezing cough quality, which could assist in distinguishing the test subject's coughs from coughs generated by other persons.

7.3.3.2 Longitudinal cough frequency measurements

Baseline assessments were conducted within 2 weeks of stroke onset and included patient demographics, stroke characteristics and respiratory parameters. Swallowing function was described according to swallow screens and clinical bedside assessments, which were conducted as part of routine acute stroke care (Appendix 2). The swallow was deemed 'unsafe' if any swallowing precautions or specific dysphagia management strategies had been specified for the person, which could include nil by mouth (NBM), modified texture diet and thickened fluids, or specific swallowing strategies (body positioning, head turn swallow, supervision by staff when swallowing, etc.). The swallow was deemed 'safe' if the patient was allowed to eat and drink normally without any precautions or instructions. Respiratory assessments at baseline were forced spirometry, maximal mouth pressure measurements and cough flow measurements of volitional and capsaicin-induced reflex cough. A detailed description of respiratory assessment methods is given in chapter 3.

LCM devices were fitted to patients at baseline; and at one week, four weeks and twelve weeks thereafter. 24-hour cough frequency counts were obtained at each of these time points. Patients were either inpatients on the acute stroke unit, or had already been discharged home at the time of recording. Patients were asked to wear the device during the entire 24-hour period,

except for when having a bath or shower. Patients were instructed that, when asleep, they should either continue wearing the device on their person, or place it close by, for example on a bedside table. These instructions and the researcher's contact details were provided to participants with an information leaflet. LCM devices were collected after the recording period, and recordings were processed by the researcher. Summary reports generated by the LCM software were printed, and data transferred to a Microsoft Office Excel spreadsheet.

7.3.4 Data analysis

Data pertaining to the validation of the LCM in this sample was analysed through assessment of the raw data and descriptive statistics; and by calculation of the ICC for absolute agreement (Streiner & Norman 2008), using Stata statistical software (Stata v12.1, StatCorp, College Station, TX) and a two-way mixed effects model for individual agreement (StataCorp 2013).

Data from longitudinal cough frequency measurements was collected on a Microsoft Office Excel spreadsheet. To allow for convenient assessment of the distribution of cough events over a 24-hour period, hourly cough counts were re-arranged so that for each participant the 24-hour period started at 00:00 hours midnight (*e.g.*, if the LCM recording had started at 10:00am and ended at 10:00am the following day, cough counts recorded from 00:00 to 10:00am the following day were cut and pasted to the beginning of the time period, thereby resulting in a 00:00 to 24:00 hours period). Descriptive statistics were used to describe hourly and total cough counts, calculating arithmetic mean, median and geometric mean. The diurnal distribution of hourly cough frequency was illustrated graphically. Repeated measures ANOVA was used to assess for within-group differences at study time points. For this analysis, square root transformation of data was conducted to obtain approximately normally distributed data. Original and square root transformed data were also used to compute 95% confidence intervals as an inferential estimate of cough frequency for the study population. To compare the stroke cohort with norm values, logarithmic transformation of data was conducted, as in the literature cough frequency data is commonly reported as geometric mean and logSD. Statistical significance test for the mean difference between two independent samples with unequal variance was used to

compare the stroke cohort with norm values from healthy subjects and subjects with respiratory disease. Relationships between cough frequency and participant characteristics were explored using Spearman's rank correlation coefficient and independent samples comparison tests. This basic approach was selected due to the small sample size and the exploratory nature of the analysis. Also, no adjustment for multiple testing was made, and this needs to be considered when interpreting the reported p-values. Analyses were conducted using Microsoft Office Excel 2007 and Stata statistical software.

7.4 Result

7.4.1 Description of the sample

Data were collected from 21 acute stroke patients between September 2012 and November 2013. Sample characteristics are summarised in Table 39. The cohort consisted of 7 women and 14 men with mean (SD) age of 60 (15) years. The majority had a moderate level of stroke impairment on admission, with a median (IQR) NIHSS score of 8 (5, 11). The aetiology of stroke was ischemic in 86% and hemorrhagic in 14%. The majority of patients had cortical (43%) or subcortical (43%) stroke lesion sites. Two patients (10%) had brainstem strokes and one (5%) had a cerebellar stroke. Approximately two thirds of patients (62%) were considered to have safe swallow function according to the routine bedside swallow assessment, and approximately one third (38%) had unsafe swallow. Seven patients (33%) had pre-existing prescriptions for ACE inhibitors, which were continued for the duration of the study. Eleven patients (52%) were non-smokers, seven (33%) were current smokers, and three (14%) had quit smoking more than five years prior to the study. During the study period, chest radiographs were taken for 13 patients (62%), no abnormalities were detected on any radiographs. The remaining eight patients did not have chest radiographs due to lack of clinical indication. Pneumonia incidence was observed for the first four weeks following baseline assessment for the entire sample, and from week four to week twelve for those patients who remained in the study. There was one participant (5%) who developed pneumonia.

Table 39. Sample characteristics at baseline

Sample characteristics at baseline	n=21
Age	60 (15)
Sex	
Female	7 (33%)
Male	14 (67%)
Stroke type	
Ischemic	18 (86%)
Hemorrhagic	3 (14%)
Stroke side	
Left-sided	12 (57%)
Right-sided	8 (38%)
Bilateral	1 (5%)
Stroke site	
Cortical	9 (43%)
Subcortical	9 (43%)
Brainstem	2 (10%)
Cerebellar	1 (5%)
Stroke severity (median (IQR) NIHSS score)	8 (5, 11)

Table 39. continued

Smoking	
Never smoked	11 (52%)
Quit >5 years ago	3 (14%)
Current smoker	7 (33%)
Swallowing status	
Safe	13 (62%)
Unsafe	8 (38%)
ACE-inhibitor use	
Yes	7 (33%)
No	14 (67%)
Chest radiograph	
Normal	13 (62%)
Abnormal	0
Not taken	8 (38%)
Pneumonia from baseline to week four	
Yes	1 (5%)
No	20 (95%)

Figures are mean (SD) and frequency (%), unless stated otherwise; ACE, angiotensin-converting enzyme; NIHSS, National Institutes of Health Stroke Scale

Baseline respiratory parameters for the sample are presented in Table 40. Lung function (forced spirometry) showed that average forced vital capacity, forced expiratory volume in one second, and peak expiratory flow was approximately 70% of the respective predicted values. Mean (SD) expiratory and inspiratory mouth pressures were 69 (40) and 48 (33) cmH_2O, respectively. Mean (SD) peak expiratory flow of voluntary and involuntary cough were 500 (255) and 259 (113) L/min, respectively.

Table 40. Summary of baseline respiratory parameters for the sample

Parameter	(n=21)
Forced spirometry	
FVC (L)	2.6 (1.2)
% of predicted FVC	70 (23)
FEV_1 (L)	2.1 (1.0)
% of predicted FEV_1	71 (24)
PEF (L/min)	315 (156)
% of predicted PEF	70 (29)
Respiratory muscle strength	
PEmax (cmH_2O)	69 (40)
PImax (cmH_2O)	48 (33)
Cough intensity	
Voluntary cough PECF (L/min)	500 (255)
Involuntary cough PECF (L/min)	259 (113)

Figures are mean (SD)

FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; PECF, peak expiratory cough flow; PEF, peak expiratory flow; PEmax; maximal expiratory mouth pressure; PImax, maximal inspiratory mouth pressure

7.4.2 Validation of the Leicester Cough Monitor in acute stroke

Potential concerns regarding the validity of the sound-based LCM system in this patient group were that, for patients who are inpatients at the time of recording, coughs from patients sharing the room, members of staff or visitors may contaminate the patient cough count and result in higher measurement; or that for patients with severely weakened or atypical sounding cough (*e.g.* 'bovine' cough sound in a patient with bulbar involvement) the LCM software would not recognise coughs and result in lower measurement. Table 41 shows the cough counts obtained during a 15-minutes period from 'live' observation by a researcher compared with cough counts obtained from the LCM device.

Table 41. Cough counts obtained during a 15-minutes period from five test subjects in a four-bedded acute hospital bay. Subjects' coughs and ambient coughs were observed and counted 'live' by a researcher who was present for the duration of the recording.

Subject	Direct observation		Automated cough count (LCM)
	Total number of coughs in the environment (subject's coughs plus ambient coughs)	Subject's coughs	
1	24	11	12
2	17	5	5
3	21	9	9
4	51	27	28
5	35	15	14
Total	148	67	68
Arithmetic mean	30	13	14
Median	24	11	12
Geometric mean	27	12	12

LCM, Leicester Cough Monitor

From an assessment of the raw data and the group totals, means and medians presented in Table 41, it is evident that there was good agreement between subjects' coughs as counted by the observing researcher and as determined from the LCM system. Based on the assumption

that the observing researcher's 'live' cough count (group total of 67) is taken as the 'true' cough count, the LCM system accurately measured subjects' cough frequency to within one cough (group total of 68). Although during the observation period a considerable number of cough sounds had been counted by the observing researcher, which had not generated from the test subject but from other persons in the environment (group total of 81), these ambient coughs did not contaminate the LCM cough frequency measurement. The particular strategy of giving operator input when processing LCM sound recordings and refining the cough counts is likely to be crucial in this context. The high level of agreement is reflected in the ICC (95% CI) of 0.996 (0.967, >0.999).

7.4.3 Longitudinal observation of cough frequency in a cohort of acute stroke patients

7.4.3.1 Description of cough frequency in the sample

Subjects' 24-hour cough frequency and average hourly cough frequency over 24 hours for each study time point are summarised in Table 42. Arithmetic mean, median and geometric mean are presented. Since the data shows a skewed distribution, median is more appropriate than arithmetic mean. The geometric mean is commonly used and reported in the literature, as data are generally transformed logarithmically for inferential statistical analysis.

The summary of cough frequency measurements shows that there was higher cough frequency at baseline (median (range) 24-hour cough frequency 118 (4, 375)), which dropped during the course of the study to 56 (1, 186) at four weeks and 34 (6, 108) at twelve weeks; although participant attrition between four and twelve weeks was high, and data from the twelve week time point is therefore less representative. Also, the wide range of observed cough counts amongst individuals is notable, from very low 24-hour cough frequency (below ten coughs) to higher counts (>300 coughs). There was a diurnal pattern, whereby cough frequency was consistently higher during day time than during night time. Figures 25 to 28 show averages and ranges for hourly cough frequency at each of the study time points. Figure 29 shows median

hourly cough frequency at baseline, week one and week four, illustrating the decrease of cough frequency with time.

Table 42. Summary of cough frequency at each time point

Cough frequency summary measure	Baseline (n=21)			Week 1 (n=20)		
	Arithmetic mean (SD)	Median (range)	Geometric mean (logSD)	Arithmetic mean (SD)	Median (range)	Geometric mean (logSD)
Cough frequency over 24 hours	130 (102)	118 (4, 375)	84 (1.2)	80 (69)	60 (6, 217)	52 (1.0)
Average hourly cough frequency for 24 hour period	5 (4)	5 (0, 16)	4 (1.2)	3 (3)	2 (0, 9)	2 (1.0)
Number of day time coughs [a]	95 (74)	86 (4, 282)	63 (1.1)	57 (53)	30 (6, 159)	37 (1.0)
Average hourly cough frequency at day time	7 (5)	6 (0, 20)	4 (1.2)	4 (4)	2 (0, 11)	3 (1.0)
Number of night time coughs [a]	34 (36)	21 (0, 112)	20 (1.3)	23 (19)	18 (0, 58)	17 (1.1)
Average hourly cough frequency at night time	3 (4)	2 (0, 11)	2 (1.3)	2 (2)	2 (0, 6)	2 (1.1)

Table 42. continued

Cough frequency summary measure	Week 4 (n=17)			Week 12 (n=5)		
	Arithmetic mean (SD)	Median (range)	Geometric mean (logSD)	Arithmetic mean (SD)	Median (range)	Geometric mean (logSD)
Cough frequency over 24 hours	66 (59)	56 (1, 186)	36 (1.5)	54 (44)	34 (6, 108)	35 (1.1)
Average hourly cough frequency for 24 hour period	3 (2)	2 (0, 8)	2 (1.2)	2 (2)	1 (0, 4)	2 (1.0)
Number of day time coughs [a]	48 (36)	41 (1, 108)	29 (1.4)	41 (33)	26 (5, 84)	27 (1.1)
Average hourly cough frequency at day time	4 (3)	3 (0, 8)	2 (1.4)	3 (2)	2 (0, 6)	2 (1.0)
Number of night time coughs [a]	18 (26)	9 (0, 90)	12 (1.2)	13 (14)	8 (0, 42)	9 (1.3)
Average hourly cough frequency at night time	2 (3)	1 (0, 9)	1 (1.2)	1 (1)	1 (0, 4)	0 (1.2)

[a] Daytime: 06:00 to 22:00; night time: 22:00 to 06:00

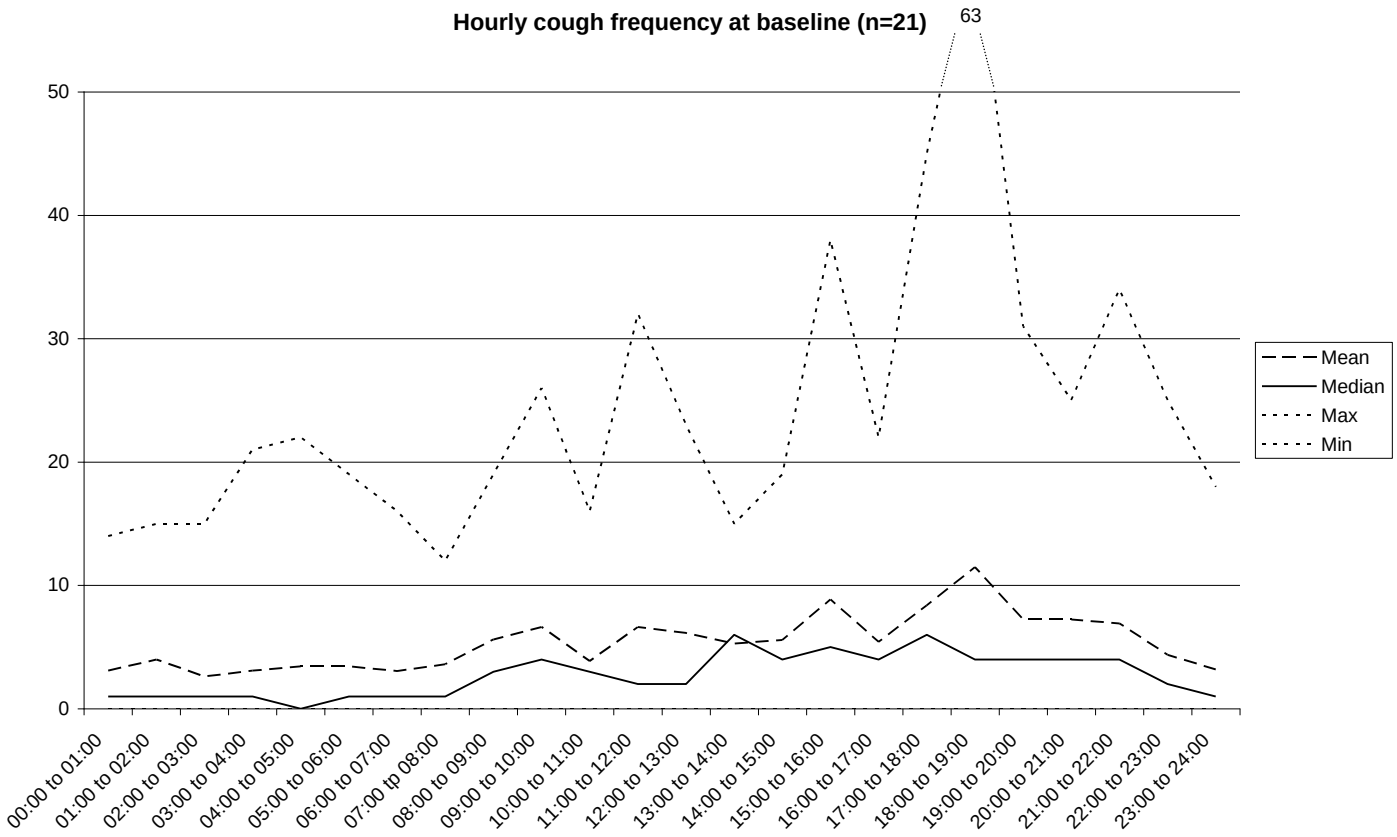


Figure 25. Hourly cough frequency at baseline. The line indicating the minimum hourly cough frequency corresponds with the x-axis, as minimum hourly cough frequencies across the entire 24 hour time period were zero.

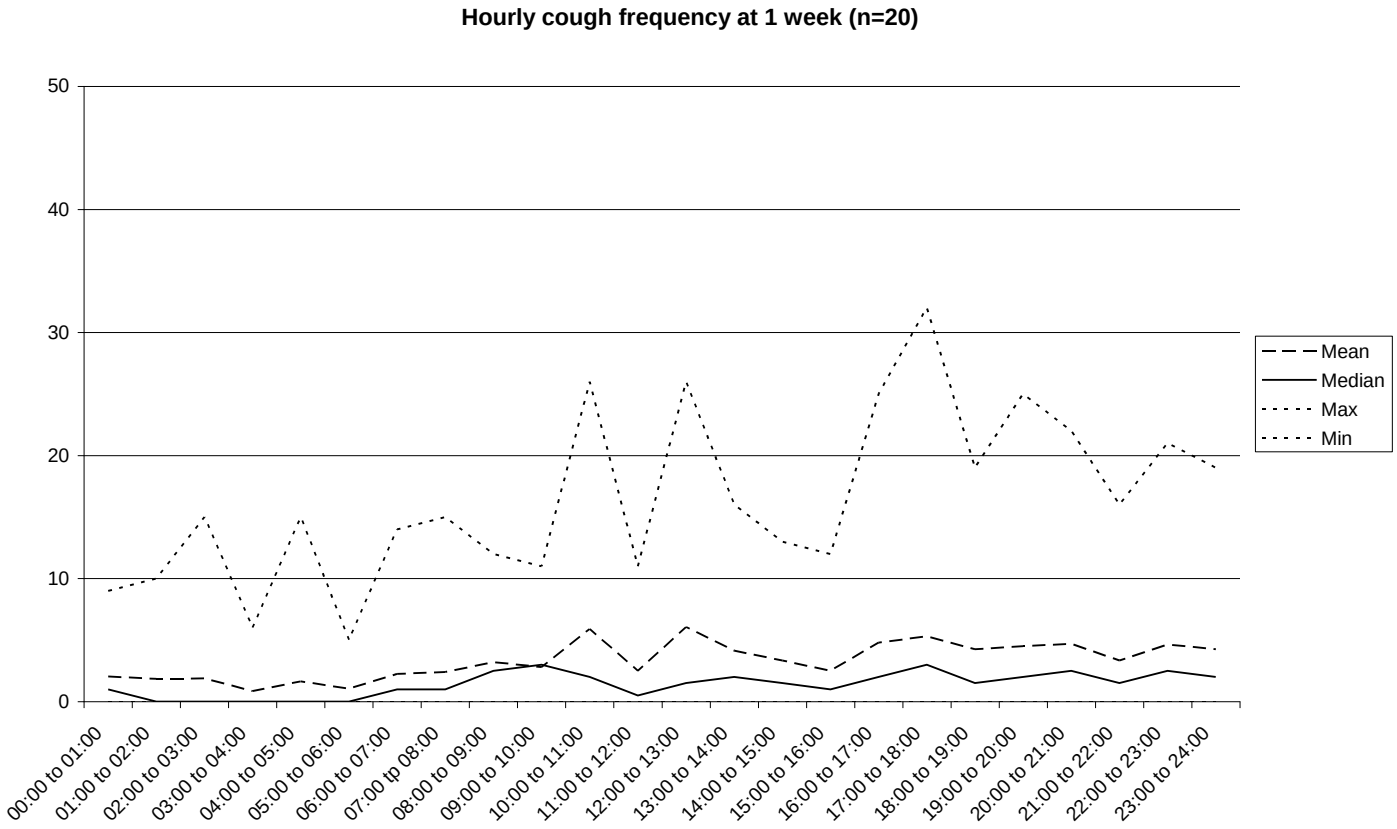


Figure 26. Hourly cough frequency at week 1. The line indicating the minimum hourly cough frequency corresponds with the x-axis, as minimum hourly cough frequencies across the entire 24 hour time period were zero. The median line overlaps with the x-axis between 01:00 and 06:00 hours.

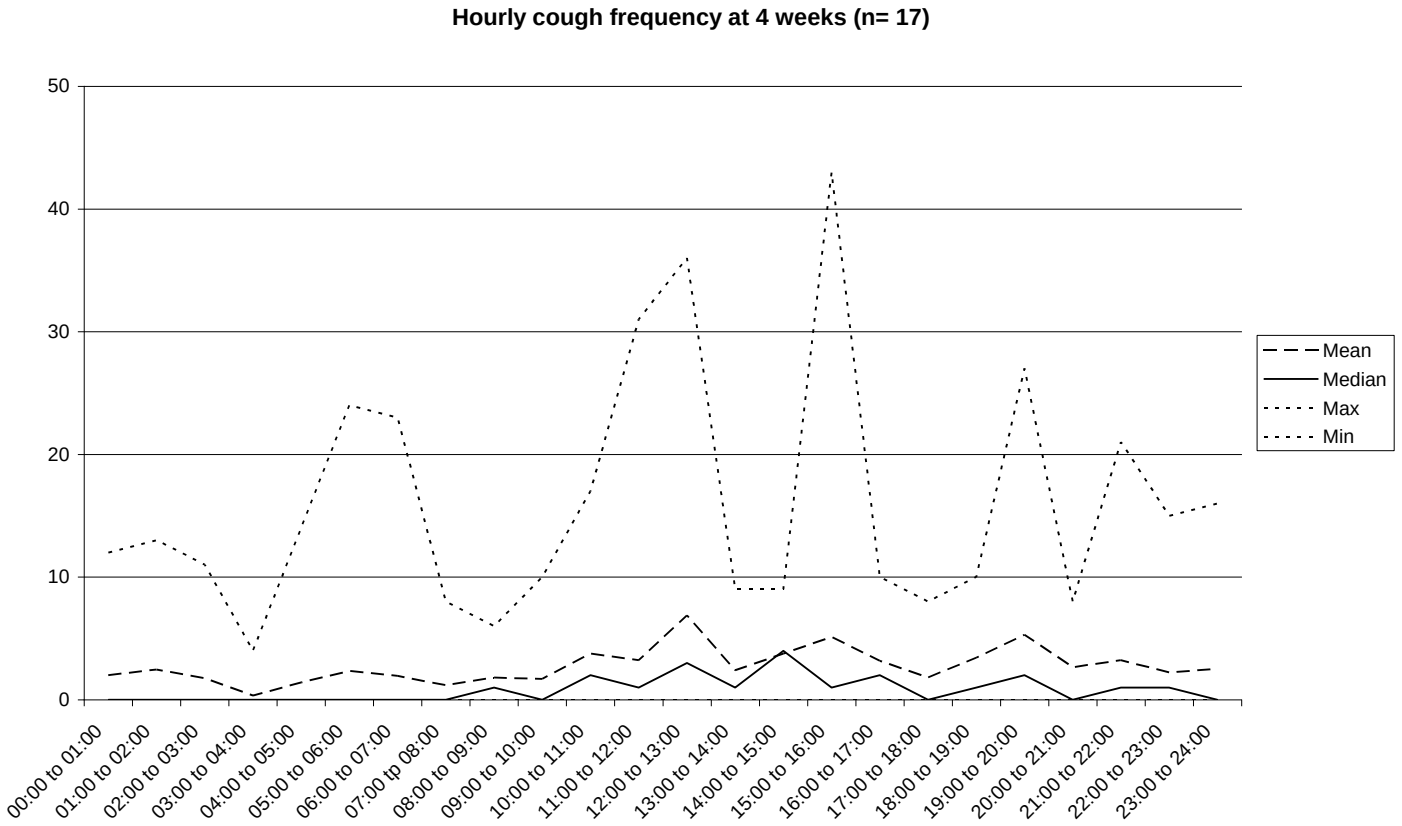


Figure 27. Hourly cough frequency at week 4. The line indicating the minimum hourly cough frequency corresponds with the x-axis, as minimum hourly cough frequencies across the entire 24 hour time period were zero. The median line overlaps with the x-axis between 00:00 and 08:00 hours.

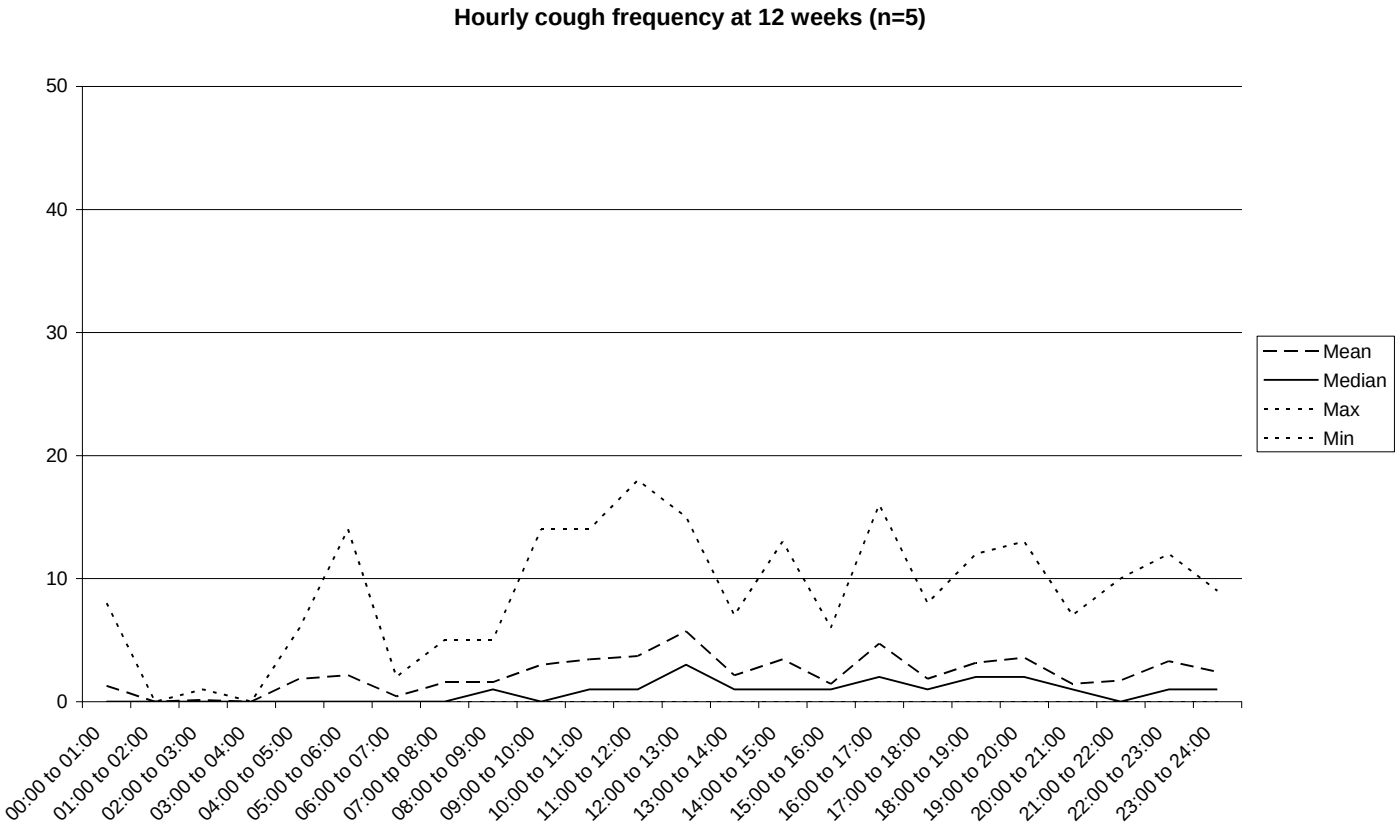


Figure 28. Hourly cough frequency at week 12. The line indicating the minimum hourly cough frequency corresponds with the x-axis, as minimum hourly cough frequencies across the entire 24 hour time period were zero. The mean line overlaps with the x-axis between 00:00 and 02:00 hours, and the median line overlaps with the x-axis between 00:00 and 08:00 hours.

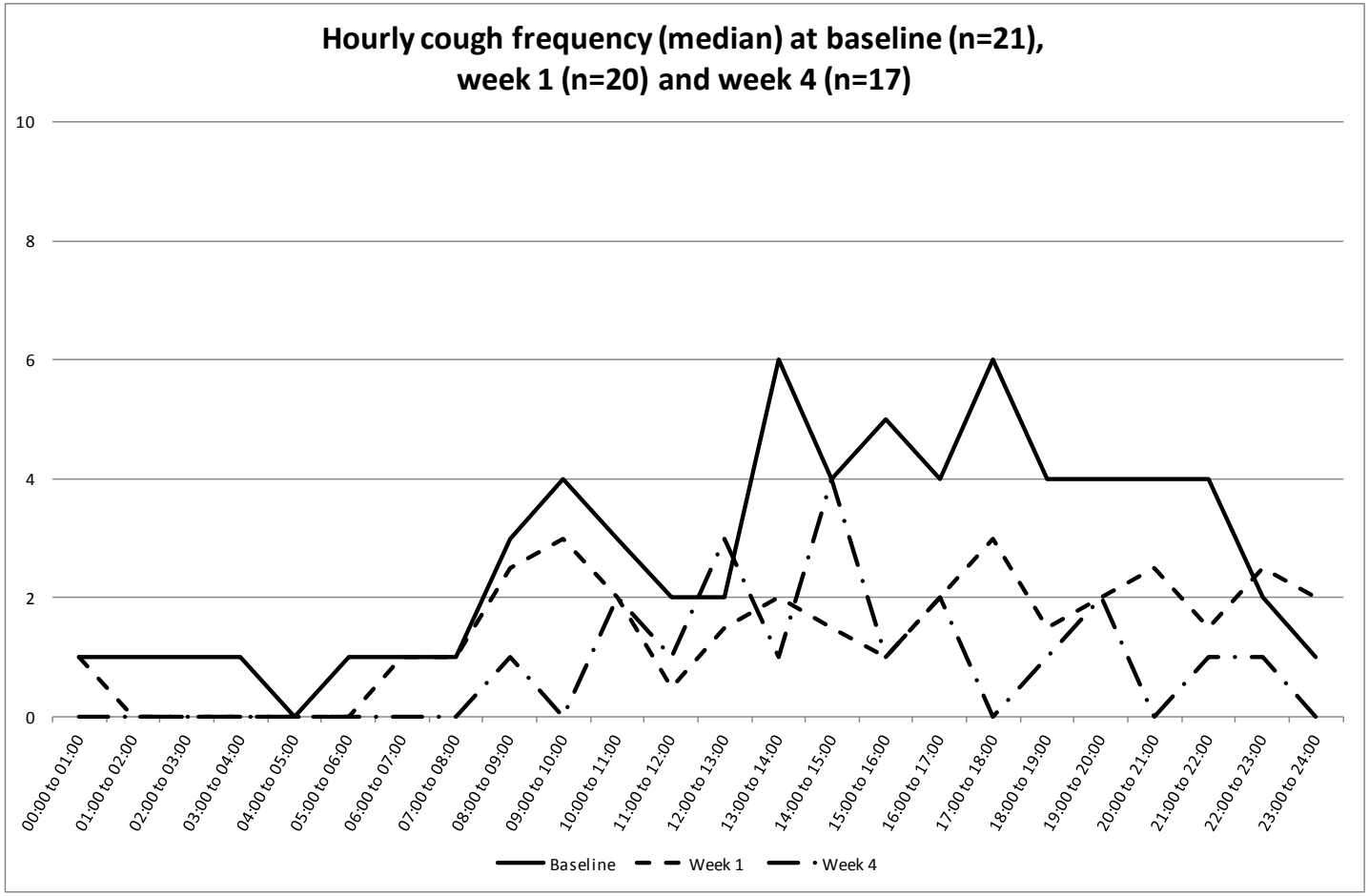


Figure 29. Hourly cough frequency (median) at baseline (n=21), week 1 (n=20) and week 4 (n=17). The graph illustrates a trend of decreasing cough frequency over time. Area under the curve (AUC) at baseline, week 1 and week 4 is 64.7, 33.3 and 20.1, respectively (p=0.0003).

7.4.3.2 Within-group comparison of cough frequency at the study time points

Within-group comparisons of 24-hour cough frequency and average hourly cough frequency confirmed that the observed decrease in cough frequency over time is statistically significant. Arithmetic mean 24-hour cough frequency at the four study time points decreased from 130 at baseline to 80 at week one, 66 at week four, and 54 at week twelve. Arithmetic mean hourly cough frequency at the study time points decreased from 5 at baseline to 3 at week one, 3 at week four, and 2 at week twelve. The respective p-values (using square root transformed data to meet normality assumptions in a repeated-measures ANOVA) were 0.0142 and 0.0153.

7.4.3.3 Estimation of cough frequency in the population from which the study sample was selected

The observed cough frequency data showed a skewed distribution, and were most successfully transformed to normally distributed data using the square root transformation. The 95% confidence intervals were calculated for 24-hour cough frequency at baseline and for average hourly cough frequency at baseline (Table 43). The 95% confidence intervals for the root transformed data have greater validity, since the normality assumption is met, but these cannot be meaningfully back-transformed to the original unit of measurement. Confidence intervals were therefore also computed for the data in original unit of measurement; however, due to the skewed distribution of the original data these estimates are likely to lack the statistical precision that could be achieved with normally distributed data. Bearing this limitation in mind, it can be concluded that in the population from which the sample came, average 24-hour cough frequency is likely to lie within the range from 83 to 176 coughs with 95% probability; and average hourly cough count is likely to lie within the range from 4 to 7 coughs with 95% probability.

Table 43. Sample mean (95% confidence intervals) for 24-hour cough frequency and average hourly cough count at baseline (n=21). 95% confidence intervals are given for the data in the original unit of measurement (counts) and for the square root transformed data.

	Original unit of measurement	Square root transformed data
24-hour cough frequency	130 (83, 176)	10 (8, 13)
Average hourly cough count	5.4 (3.5, 7.4)	2.1 (1.7, 2.6)

7.4.3.4 Comparison of cough frequency in acute stroke with healthy subjects and subjects with respiratory disease

Reference values for 24-hour cough frequency are reported by Yousaf *et al*. (2013). The geometric mean (logSD) 24-hour cough frequency was 18.6 (0.5) in a sample of 44 healthy non-smokers, and 33 (0.6) in a group of 6 healthy smokers. The stroke sample in the present study had geometric mean (logSD) 24-hour cough counts of 84 (1.2) at baseline, 52 (1.0) at week one, 36 (1.5) at week four, and 35 (1.1) at week twelve. Compared with healthy non-smokers, the stroke cohort had significantly higher 24-hour cough frequency at baseline and week one with p-values of <0.0001 and 0.0002, respectively. At weeks four and twelve, the differences were no longer statistically significant with p-values of 0.093 and 0.27, respectively. Baseline 24-hour cough frequency was also significantly higher in the stroke group compared with healthy smokers (p=0.018), but not at the later time points. Reported 24-hour cough frequency in groups with respiratory disease ranges from geometric mean (logSD) of 106 (0.7) in bronchitis (n=5) to 477 (0.3) in patients with unexplained chronic cough (n=34) (Yousaf *et al*. 2013). Cough frequency in the present stroke sample was therefore lower than in groups with respiratory pathologies, and higher than in healthy non-smokers and smokers within the first three weeks of stroke onset, with a trend towards normal values thereafter.

Of note, there was a difference in 24-hour cough frequency between stroke patients who were using ACE inhibitors (baseline geometric mean (logSD) 119 (0.7), n=7) and stroke patients who were not (baseline geometric mean (logSD) 70 (1.3), n=14), although this difference was not statistically significant (p=0.25). The cough frequency of stroke patients taking ACE inhibitors in was similar to the geometric mean (logSD) cough frequency of 128 (0.2) reported for a group of 4 healthy subjects using ACE inhibitors (Yousaf *et al*. 2013).

7.4.3.5 Relationship between cough frequency and other parameters in acute stroke

Baseline 24-hour cough frequency and average hourly cough count at baseline were examined in relation to patient characteristics (sex, age, stroke site, stroke severity and swallowing safety) and respiratory parameters at baseline (lung function, respiratory muscle strength, cough flow).

There was no statistically significant difference in baseline cough frequency between male and female participants (Table 44). There was no correlation between age and cough frequency (r_s=0.08) or between stroke severity (NIHSS score on admission) and cough frequency (r_s =0.09), and correlation coefficients were not statistically significant with p-values of 0.72 and 0.69, respectively.

Table 44. Baseline cough frequency according to sex

		Male (n=14)	Female (n=7)	p-value [a]
24-hour cough frequency	Arithmetic mean (SD)	121 (96)	147 (120)	
	Median (range)	109 (8, 357)	119 (4, 375)	
	Geometric mean (logSD)	82 (1.1)	87 (1.1)	0.92
Average hourly cough frequency [b]	Arithmetic mean (SD)	5 (4)	6 (5)	
	Median (range)	6 (<1, 15)	5 (<1, 16)	
	Geometric mean (logSD)	3 (1.5)	4 (1.4)	0.86

[a] Mann-Whitney test using log-transformed data

[b] Average hourly cough frequency during 24 hours

Baseline cough frequency varied according to stroke lesion site (Table 45) with statistically significant differences, although these data need to be interpreted with caution due to low numbers of subjects with brainstem (n=2) and cerebellar stroke (n=1). The highest cough frequency was observed in the two patients with brainstem strokes, with 24-hour cough counts of 225 and 357, followed by patients with cortical stroke (median (range) 24-hour cough frequency of 123 (4, 375)). The lowest cough frequency was seen in patients with subcortical stroke lesions (median (range) 24-hour cough frequency of 72 (8, 141)).

Table 45. Baseline cough frequency according to stroke lesion site

		Cortical (n=9)	Subcortical (n=9)	Brainstem (n=2)	Cerebellar (n=1)	p-value [a]
24-hour cough frequency	Arithmetic mean (SD)	155 (108)	69 (44)	291 (93)	127 [c]	
	Median (range)	123 (4, 375)	72 (8, 141)	291 (225, 357)		
	Geometric mean (logSD)	102 (1.3)	50 (1.0)	283 (0.3)		0.0496
Average hourly cough frequency [b]	Arithmetic mean (SD)	6 (4)	3 (2)	12 (4)	5 [c]	
	Median (range)	5 (<1, 16)	3 (<1, 6)	12 (9, 15)		
	Geometric mean (logSD)	4 (1.2)	2 (1.0)	12 (0.3)		0.0496

[a] Kruskal-Wallis test using log-transformed data

[b] Average hourly cough frequency during 24 hours

[c] One observation only

There was no difference in 24-hour cough frequency or average hourly cough count between patients with safe and unsafe swallow (Table 46). There were weak to moderate inverse correlations between baseline respiratory parameters and cough frequency. Spearman's rank correlation coefficients for the correlations between FVC, FEV_1, PEF, PEmax, PImax, voluntary cough PECF and involuntary cough PECF were -0.26 (p=0.25), -0.30 (p=0.18), -0.16 (p=0.49), -0.13 (p=0.57), -0.41 (p=0.065), -0.02 (0.94) and -0.13 (p=0.56), respectively.

Table 46. Baseline cough frequency according to swallow safety

		Safe swallow (n=13)	Unsafe swallow (n=8)	p-value [a]
24-hour cough frequency	Arithmetic mean (SD)	136 (104)	118 (106)	
	Median (range)	119 (4, 375)	112 (8, 357)	
	Geometric mean (logSD)	87 (1.2)	79 (1.2)	0.85
Average hourly cough frequency	Arithmetic mean (SD)	6 (4)	5 (4)	
	Median (range)	5 (<1, 16)	5 (<1, 15)	
	Geometric mean (logSD)	4 (1.2)	3 (1.2)	0.81

[a] Mann-Whitney test using log-transformed data

7.5 Discussion

The present study was the first to explore cough frequency in a sample of acute stroke patients. For this purpose, the LCM cough frequency measurement system was validated for use on an acute stroke unit. A high ICC of 0.996 was observed. When interpreting the magnitude of ICC

values, a distinction is made with respect to the use of a measurement. When a measurement is made for the purpose of individual clinical judgement, discrepancies between individual measurements have greater influence and reliability should therefore be very high (*e.g.* ICC of ≥0.90). When a measurement is made for the assessment of group averages, as is the case in the present research, the sample size serves to reduce the error of measurement and lower reliability coefficients are acceptable (*e.g.* ICC ≥0.70) (Streiner & Norman 2008, pp. 194-5). Accordingly, the present data suggests that cough counts using the LCM system are accurate for the purpose of individual and group average measurement in acute stroke unit environments. A limitation of the validation method used was that it relied on accurate 'live' cough counts by the observing researcher, which were taken as the 'true' cough counts for analysis and could not be verified at a later time. An alternative would have been to video-record the participant during the entire time of cough monitoring, which would have allowed for retrospective verification of subjects' actual cough frequency. From the validation study it was also noted that, for the application of the LCM in neurological populations where cough sounds can be atypical (*e.g.* 'bovine' cough sounds in patients with bulbar involvement), it may be useful to provide the software operator with a sound sample of the individual subject's cough as a reference example; and/or to refine the LCM system's cough sound library according to these atypical cough sounds.

For the cohort study, a convenience sample of 21 acute stroke patients was recruited. In this sample, average cough frequency within two weeks of stroke was approximately four times higher than in healthy non-smokers, and reduced over the following twelve weeks to approximately twice the level of healthy non-smokers. There was great variation in individual cough frequency, from values in the low range of normal cough frequency to values seen in the higher range of individuals with respiratory pathology. Cough frequency was approximately twice as high in cortical stroke patients as it was in patients with subcortical stroke lesion. Cough frequency in patients with brainstem and cerebellar stroke was also high, but these measurements are limited due to the small number of patients (n=3). There were no associations between cough frequency and sex, age, stroke severity or respiratory parameters. There was no difference in cough frequency between patients who were assessed to have safe

swallow, and patients whose swallow was assessed to be unsafe. One participant developed pneumonia during the study period.

The present study is limited due to the small sample size. The data have been submitted to multiple exploratory statistical analyses, which were unadjusted for multiple comparison and lacked adequate statistical power. These results, in particular regarding relationships between cough frequency and patient characteristics, therefore need to be interpreted with caution. Two observations appear reasonably valid and merit further discussion: high cough frequency soon after stroke, which gradually decreases with time; and few instances of very low cough frequency.

First, if the presence of cough and increased cough frequency are interpreted as potential signs of dysphagia (as is the case in clinical swallowing assessment), the observed reduction of cough frequency with time corresponds with the good potential for spontaneous improvement of dysphagia in the short term following stroke (Smithard *et al*. 2007, Singh & Hamdy 2006). There was no difference in cough frequency between patients with safe and unsafe swallow; although this may be confounded due to the small sample, potential subclinical levels of dysphagia undetected by BSA, and the use of ACE inhibitors by one third of participants. ACE inhibitors are known to sensitise the cough reflex and increase cough frequency. Participants who used ACE inhibitors had increased cough frequency similar to healthy subjects who use ACE inhibitors. ACE inhibitors in these patients were clinically indicated, and ethically it was not possible to alter these prescriptions. However, those participants who did not use ACE inhibitors still exhibited cough frequencies that were over three times higher than normal values for healthy subjects. Cough frequency may also be increased due to respiratory pathology. This study excluded patients with pre-existing respiratory disease, and chest radiographs were conducted in two thirds of patients during the study period to exclude respiratory disease (chest radiographs were not clinically indicated in the remaining patients during the study period). It may therefore be assumed that the high cough frequency observed was not related to the presence of respiratory disease.

Second, the majority of patients showed no dampening of the cough reflex, as most participants had normal or increased cough frequencies. Only three participants (14%) exhibited very low cough frequencies of less than one hourly coughs. Cough frequency in this study sample may be interpreted as an indicator of reflex cough sensitivity, based on the assumption that coughs are triggered mechanically to protect from potential aspiration of saliva, food or drink; and based on the exclusion of patients with respiratory pathology, which could otherwise cause increased cough frequency. If very low cough counts are taken as an indication of dampened cough reflex, the incidence observed in the present study (14%) corresponds with the findings from Addington *et al*. (2005, 1999a), who tested reflex cough through inhaled L-tartaric acid and reported a diminished or absent cough reflex in 11% (112 out of 979) of acute stroke patient.

Only one participant developed pneumonia during the study period, which does not provide statistically useful data. This patient was a 78 years old male with a subcortical stroke and admission NIHSS score of 12. This patient's cough frequency was at the lower range of normal, with 24-hour cough counts around 30 and hourly cough frequencies of one to two. In future studies, it may be of interest to examine cough frequency as a predictor of pneumonia incidence after stroke. Addington *et al*. (2005, 1999a) have shown that diminished or absent reflex cough is associated with increased risk of post-stroke pneumonia. However, their method of assessing the cough reflex through inhaled L-tartaric acid requires the patient to perform an adequate volitional inhalation, to ensure appropriate delivery of the irritant to the broncho-laryngeal receptor sites. This can be difficult to achieve in patients with cognitive impairment or limited co-operation. The LCM, as a non-invasive tool that requires minimal patient effort, may offer a convenient alternative to inform about reflex cough in this patient group.

The distribution of cough frequency over the day, in particular in relation to ingestion of food and drink, may identify an interesting area for future research. The diurnal distribution of cough frequency in the present study (*i.e.* higher cough counts during waking hours, lower cough counts during night time) corresponds with data from healthy subjects (Yousaf *et al*. 2013). In the present study, the times at which participants had food or drink were not captured. It was therefore not possible to compare cough frequency at times of ingesting food or drink with

cough frequency in between the ingestion of food or drink. In the context of neurogenic dysphagia and cough as protection from aspiration, a study of this interaction may inform about the relative importance of aspiration risk during ingestion of food and drink (hypothesising that high cough frequency will coincide with mealtimes) versus ongoing micro-aspiration of saliva (hypothesising that cough frequency in between mealtimes will be similar to when food and drink are ingested).

7.6 Conclusion

The present study explored longitudinal cough frequency measurements in a cohort of 21 acute stroke patients. For this purpose, the LCM was validated for cough frequency measurement in an acute stroke unit environment. The device showed a high level of accuracy for both individual and group level cough counts.

24-hour cough frequency within the first two weeks of stroke was found to be four times higher than in healthy non-smokers. Cough frequency steadily reduced with time to a level twice as high as in healthy non-smokers at twelve weeks post baseline. There was wide individual variation in cough frequency from normal values, as seen in healthy subjects, to very high values, as seen in patients with respiratory disease. Only three patients had cough frequencies lower than those in healthy non-smokers.

The study is limited due to the small sample size, and findings therefore need to be interpreted with caution. Use of ACE inhibitors by some patients may have introduced bias towards higher cough frequency; but exclusion of patients with pre-existing respiratory disease eliminated respiratory causes for increased cough counts. This exploratory study is the first study to describe cough frequency in acute stroke. These data provide a basis for hypothesis-generation and may serve further study of cough frequency in the context of neurogenic dysphagia and aspiration pneumonia.

Chapter 8 Discussion

The course of studies described in this thesis aimed to investigate RMT as an intervention for improving respiratory muscle strength and cough effectiveness in acute stroke, in the context of preventing pneumonia. Previous research has provided evidence for the problem of aspiration-related pneumonia in the first weeks after stroke, and for the impairment of cough in acute stroke. The rationale for the present research was that RMT might improve cough effectiveness and lead to increased protection from aspiration, thus lowering incidence of pneumonia in acute stroke patients.

The present study applied maximal mouth pressure measurements as markers of respiratory muscle strength; and cough flow measurements as parameters of cough effectiveness. Depending on study settings, accuracy and reproducibility of these volitional respiratory assessments can be limited. For the present research, methodological aspects of these assessment procedures were therefore investigated in-depth. PECF was the primary outcome of interest. The accuracy of different instruments when measuring PECF was assessed to inform the selection of the most appropriate measurement device (chapter 4). Good instrument performance of the study mouth pressure meter and cough flow measurement system was demonstrated in a series of laboratory bench tests. It was shown that test-retest reliability in humans was good and of equivalent magnitude in healthy volunteers and in stroke patients, demonstrating that these respiratory assessments could be performed as reliably in the patients enrolled in the research as in healthy subjects (chapter 3).

These measurement techniques were used to investigate the effectiveness of RMT for improving cough flow in a single-blind randomised placebo-controlled trial, which included 82 patients in three study arms (expiratory muscle training, inspiratory muscle training and sham respiratory training; chapter 5). The trial was novel in that it investigated RMT in the acute phase of stroke and with the aim of improving cough flow. RMT was shown to be safe, well tolerated and a low-cost intervention in this patient group. However, training at the frequency

and intensity as applied in this study was not effective in increasing cough flow or maximal mouth pressures when compared with sham training. In a secondary analysis of trial data, cough flow was examined as a predictor of four-week pneumonia incidence (chapter 6). Although the incidence of PSP was not different in study groups, the risk of PSP decreased in proportion with increasing PECF in stroke patients who were at risk of aspiration, but not in patients with safe swallow. This confirms that stronger cough protects from aspiration-related pneumonia after stroke. PECF of volitional cough was a stronger predictor than PECF of reflex cough, and it may be useful to assess voluntary PECF in clinical practice to categorise patients according to PSP risk.

Lastly, cough frequency was studied longitudinally in a cohort of 21 acute stroke patients. This was the first study of cough frequency in acute stroke. For this purpose, an automated cough frequency measurement system was validated for use in an acute stroke unit environment. 24-hour cough frequency within the first two weeks of stroke was found to be four times higher than in healthy non-smokers. Twelve weeks later, cough frequency had reduced to a level twice as high as in healthy non-smokers. Individual variability in cough frequency was wide, and cough frequency did not correlate with sex, age, stroke severity, respiratory function, cough intensity, or swallow safety. These data provide a basis for hypothesis-generation and may serve further study of cough frequency in the context of neurogenic dysphagia and aspiration pneumonia.

8.1 Potential sources of bias and limitations

8.1.1 Internal validity

8.1.1.1 Passage of time

The work presented in this thesis was conducted over the period of several years. History can present a threat to the internal validity of studies which run over an extended period of time (Domholdt 2005, pp. 86-88); although this would potentially have more impact on the observational study of cough frequency (chapter 7) than on the randomised controlled trial of RMT (chapter 5). There was no significant historical event, such as a major change in stroke care provision at the study site, during the data collection period. The most significant recent innovation in UK stroke care has been the introduction of hyper-acute stroke units with routine provision of thrombolysis for ischemic stroke. This was introduced at the study site in 2008, well before the present research took place. Rehabilitation provision can change over time to adapt to local requirements, but there was no re-structuring of inpatient or community stroke rehabilitation services at the study site during the study period.

Another potential threat to internal validity related to the passage of time is that study instruments may be affected through wear and tear, or that instruments may be switched during the course of research, which may lead to skewed findings (Domholdt 2005, p. 91). For the present studies, the same instruments were used throughout the data collection period, and regular calibration checks were conducted to ensure stable instrument performance (chapter 3).

8.1.1.2 Repeated testing

Repeated testing, and in particular repeated performance of volitional tests, may impact on internal validity (Domholdt 2005, pp. 89-91). It is possible for a measured change to occur due

to familiarisation with the testing procedure, rather than the intervention stimulus. This point has been raised in the context of using maximal mouth pressure measurements as outcomes in studies of RMT (Polkey & Moxham 2004). One method of controlling for this threat to validity is the inclusion of a control group, which completes all assessment procedures in the same way the intervention group does, and this method was applied in the randomised trial of RMT (chapter 5). The overall improvement that was observed in all study groups may therefore be attributed to spontaneous post-stroke recovery, familiarisation with the volitional tests (*i.e.* participants became better at performing test procedures), or a combination of both. To inform the potential for measurement change due to repeat testing, the volitional respiratory assessment methods applied in the present study were examined for test-retest reliability in the absence of 'true' change (chapter 3). Healthy volunteers and stroke patients repeated the tests in the space of two hours. These data were important to inform about the expected magnitude of measurement variability in the absence of change; and to interpret the magnitude of differences in pre- and post-intervention tests.

Alternative methods to control for the effect of repeated testing have been described (Domholdt 2005, pp. 89-91). In a controlled study design, it is possible to conduct a post-intervention test only; although this eliminates the possibility of comparing study groups at baseline for relevant differences in the outcome parameter. A familiarisation period can be planned at the study start, with the aim to achieve stable repeat test performances by participants before the intervention is introduced. This method is more suitable for study populations which are stable within their condition and where timeliness is not a factor. This approach was not possible in the present study, which relied on recruitment of participants and implementation of the intervention as early as possible after stroke, in order to meet the clinical aim. Lastly, measurement of physiological or biological markers through non-volitional tests may offer alternatives or correlates to volitional repeat tests. In the field of respiratory muscle assessment, the diaphragm and abdominal muscles can be stimulated involuntarily through trans-cutaneous magnetic or electric stimulation. Pressure measurements from gastric and oesophageal balloon catheters during stimulation can inform about the function of respiratory muscles. The obvious disadvantages of these specialist methods are patient discomfort from the invasive balloon catheter, equipment

requirements and investigator expertise (American Thoracic Society (ATS) & European Respiratory Society (ERS) 2002, pp. 528-547).

8.1.1.3 Peak cough flow as a surrogate endpoint

In the present trial of RMT, the primary outcome peak cough flow was used as a physiological surrogate endpoint in lieu of the actual clinical outcome of interest, pneumonia. This allowed for adequate statistical power with a smaller sample size than if the study would have been powered for outcome pneumonia (section 5.5). Peak cough flow was selected based on a reasoned assumption and on evidence from a mechanical model (section 1.3.3). A retrospective secondary analysis of trial data contributed to validating the association between peak cough flow and PSP risk (chapter 6). Nevertheless, it is acknowledged that the use of surrogate outcomes can be problematic; and that findings based on surrogate outcomes should be interpreted with caution when drawing conclusions with respect to clinical endpoints (Joffe & Greene 2009, Goetzsche *et al*. 1996). Although surrogate endpoints may be selected based on sound logic and theory and on established statistical association (for example statistical evidence of a predictor-outcome association), past research provides examples where improvement in surrogate measures did not correspond with beneficial clinical outcomes (Goetzsche *et al*. 1996, D'Agostino 2000).

The parameter PECF relates closely to the mechanics of cough and protection from aspiration, and on this basis it was selected as the surrogate measure for the present research. However, other physiological surrogate measures relating to cough function and cough effectiveness may be considered. Inspiratory and expiratory maximal mouth pressures, forced vital capacity and peak expiratory flow have been examined as indicators of cough strength in a small number of studies (Boitano 2006). These measurements, as well as a number of other standardised respiratory function assessments, may potentially correlate well with cough effectiveness; and may potentially pose fewer challenges with respect to measurement instruments and standardised measurement techniques than the assessment of peak cough flow. However, there has been insufficient investigation of this.

Further, cough aspects other than peak cough flow may warrant consideration. For example, in the present study PECF of maximal volitional cough was measured, which is comparable to a 'personal best' performance. This, however, may not be the functionally relevant aspect in the context of protection from continuous aspiration threat. It may be that the capacity to consistently and repeatedly produce a certain minimum level of expiratory cough flow may constitute a more relevant measure. Non-volitional measures of cough strength over an extended period of time, as opposed to volitional 'one-off' measurements, may be more appropriate in this context. Long-term cough sound recordings with evaluation of sound pressure levels may offer an alternative measurement method, although the relevant technology requires further development and validation at this time.

8.1.1.4 Interpreting the lack of response to RMT

In the randomised controlled trial of RMT, no intervention effect was observed in comparison with sham training, and all groups improved with time. As discussed above, this improvement with time could be related to the effect of repeated testing. Alternatively, it could also be interpreted as spontaneous recovery, which usually occurs to some degree in the first weeks following stroke (Cramer 2008). Lack of response to RMT could also be related to poor training completion in the intervention groups. However, while training concordance was variable across the entire sample, it was also balanced in all study groups. Training completion was incorporated as a co-variate in the intention-to-treat analysis of the trial (section 5.3.3); and correlation analysis of change in outcome according to training completion provided evidence of weak correlations only (section 5.3.9). Overall, this supports the interpretation that the lack of demonstrated intervention effect was due to lack of response to training, as opposed to confounding through inadequate training completion.

A further possibility for the lack of response to RMT is that RMT may have been delivered with an inadequate 'active ingredient'. In the present trial, the 'active ingredient' in the intervention

was the training intensity (*i.e.* the resistance threshold), which was 50% of maximal mouth pressure in the training groups, and 10% of maximal mouth pressure in the control group. Training frequency and duration were the same in all study groups. The training parameters selected for the present study lie within the range of parameters applied in previous studies of RMT in neurological populations; although training duration was one of the shortest and intensity and frequency of training were among the highest (section 1.5.3.2). This mirrored the aim of training (*i.e.* improving cough, which occurs in short bouts of high intensity; as opposed to, for example, improving cardio-respiratory endurance, which requires capacity at lower intensity over a longer period of time). While, based on training protocols applied in previous research, the RMT protocol in the present study could reasonably have been expected to produce an intervention effect, it may be necessary to deliver RMT at even higher frequency and/or intensity to achieve improvements in respiratory muscle strength and cough flow in acute stroke; although this would likely have implications on the feasibility and completion rate of such a training protocol.

Another possibility to account for the lack of response to RMT is that the observed improvement with time in the present trial could also be related to improvement due to focussed breathing through a device. This was conducted by participants in all groups, including the sham training group. In other words, it is possible that daily focussed breathing through the device, irrespective of the threshold level, may have induced improvement. One week of daily volitional breathing exercise (diaphragmatic breathing with bio-feedback) in healthy subjects has been shown to induce plasticity of cortico-spinal diaphragm control, leading to larger cortical representation of the diaphragm (Demoule *et al*. 2008). A similar mechanism may have contributed to the findings of the present trial. To investigate this aspect, a factorial study design could have been considered (Domholdt 2005, pp. 128-134), whereby different treatment conditions and interactions between factors could have been compared against each other. Such a study design could have included a ‘no respiratory training’ control group, as well as a sham training group, and several intervention groups with differing training protocols to compare different proposed ‘active ingredients’ of training.

Lastly, the lack of response to RMT in the present trial may be considered in the context of the physiology of cough. As has been discussed in the introduction to this thesis (section 1.3.4), the rationale for trialling inspiratory muscle training was to improve pre-cough inspiratory volume. Higher pre-cough inspiratory volume increases the potential for high expiratory cough flow, by generating volume in the lungs that can then be expelled. In addition, greater inspired volume optimises the length-tension relationship of expiratory muscles and allows for higher intra-thoracic pressure to be built up during the compression phase of cough (American Thoracic Society (ATS) & European Respiratory Society (ERS) 2002, p. 535). The rationale for expiratory muscle training was to maximise intra-thoracic pressure during the compression phase of cough. Glottis function was not addressed in the present study. Intrinsic laryngeal muscle function is important in cough, as glottis closure and opening need to function effectively and in co-ordination with in- and expiratory muscle action. The rationale for not targeting glottis function in the present trial was that in previous physiological studies, glottis function in acute stroke patients did not seem to differ from matched healthy control subjects (section 1.3.4). This judgement was based on visual analysis of cough time-flow traces, whereby the presence of a glottis compression phase (trace levels at zero flow between inspiratory and expulsive phase of cough) and its duration were assessed. It is possible that not incorporating glottis function in the training intervention may account for the lack of effect on cough flow, and further work may be warranted to explore the function and co-ordination of intrinsic laryngeal muscles during cough in stroke patients in more depth. Also, an alternative training intervention that involves glottis function, either in isolation or in combination with other training components, may be considered, such as coughing with bio-feedback or similar.

8.1.2 External validity

External validity, or generalisability, of the present research can be considered with respect to the study sample, setting and time (Domholdt 2005, pp. 99-101).

8.1.2.1 Sample selection

The sample selection criteria for the present studies were shaped by two main considerations. First, participants had to be able to perform volitional respiratory manoeuvres, which excluded patients unable to follow instructions. From the point of view of implementing the RMT intervention, this first condition was necessary, as respiratory training requires the patient to perform co-ordinated volitional respiratory manoeuvres. This criterion therefore in the widest sense defined the potential target population for RMT in acute stroke. The second consideration in selecting the study sample was to exclude patients with pulmonary co-morbidities and acute cardiac events. The rationale for this was mainly related to patient safety, as repeated forceful respiratory manoeuvres and intra-thoracic pressure changes may aggravate airway obstruction or acute cardiac conditions. In addition, patients with asthma and chronic obstructive pulmonary disease can experience expiratory flow limitations due to airway obstruction (Loudon & Shaw 1967). In these patients, it can be difficult to judge the relative contributions of airway obstruction versus respiratory muscle weakness to reduction or improvement in cough flow. Had the RMT intervention shown promise, findings could not have been generalised to these patient sub-groups. For the data on cough frequency, the exclusion of patients with pulmonary co-morbidities strengthened the findings, as the risk of confounding due to known increased cough frequency in respiratory conditions was minimised.

Systematic differences between participating and non-participating eligible patients can potentially introduce bias and limit study generalisability. Although non-participation amongst eligible patients in the present research was high (57%), implications on generalisability of findings are likely negligible. From the eligibility screening criteria, there were no systematic differences between eligible patients who decided to take part and those who declined. High rates of non-participation would potentially present a greater threat to external validity if study results were in favour of the training intervention, as research volunteers may be more motivated to participate in training, particularly if there is an element of self-completed exercise (Domholdt 2005, p. 100).

Two key sample characteristics were a wide range of stroke severities (median NIHSS score 8, range 5-25) and a PSP incidence of 13%, which corresponds with PSP rates on NHS stroke units (16% in 2008 and 13% in 2010) (Royal College of Physicians 2011, 2009). In this respect, the study sample constituted a good representation of the potential target population with respect to the clinical aim of the research, which was to implement RMT as a routine preventive intervention early after stroke.

8.1.2.2 Study setting

The present trial of RMT was designed for the hyper-acute and acute stroke care pathway in UK NHS settings. Delivery of the RMT intervention was designed taking a pragmatic and feasible approach for this setting (section 5.5). Acute stroke care delivered through private sector healthcare providers or in other countries may differ in terms of patient characteristics, organisation of care (*e.g.* availability and duration of sub-acute inpatient rehabilitation provision as opposed to early supported discharge services), and availability of resources and staff time. As one example, some delivery models in continental Europe and in the USA favour early stroke rehabilitation in inpatient settings. Patients are admitted to specialised inpatient rehabilitation units once they are able to tolerate participation in three hours of daily rehabilitative therapies. Compared to an early supported discharge model, an inpatient rehabilitation setting may allow for higher levels of staff supervision during RMT and thereby promote optimal training completion (and potentially achieve better training concordance than in the present trial). At the same time, admission criteria to such an inpatient unit screen out the less 'rehab-ready' patients, who are also more vulnerable to PSP, which might impact on the clinical relevance of PSP and the use of RMT as a preventive intervention. Overall, the present research has good generalisability to other NHS hyper-acute and acute stroke care settings; however, its relevance (with respect to both the clinical rationale and the study design) to other settings will likely depend on the particular local context.

8.1.2.3 Time

The time period of the present research may be considered with respect to external validity, in particular regarding the rationale for the research. Cough as a treatment target for PSP prevention strategies has a place in the context of current knowledge and evidence; however, currently ongoing investigations of different novel approaches to PSP prevention (section 1.2.4) may lead to successful alternative clinical strategies in the future, which may render the approach pursued in the present work redundant.

8.2 Practical considerations

Recruitment and retention of research participants in the acute phase of stroke can be challenging, due to the nature of the condition and due to a dynamic, at times intense clinical service pathway. An appreciation of these challenges is helpful in the planning and conduct of clinical research (Bell *et al*. 2008, Blanton *et al*. 2006). Rates of recruitment and attrition in stroke rehabilitation research can vary widely. Investigators have reported recruitment rates (proportion of patients screened) of 6% (Blanton *et al*. 2006), 18% (Bernhardt *et al*. 2008), and 51% (Lloyd *et al*. 2010), and attrition rates of 2% (Lloyd *et al*. 2010), 27% (Bernhardt *et al*. 2008), and 39% (Blanton *et al*. 2006). These variations are likely due to multi-factorial reasons, including the time from stroke onset and the length of the trial. In comparison, the present trial of RMT (chapter 5) had the lowest recruitment rate at 4.5%. Attrition was fair at the first re-assessment at four weeks (23%), and high at the second re-assessment at twelve weeks (54%).

In the present trial of RMT, time afforded for study procedures was given as one of the most frequent reasons to decline participation (19% of eligible patients). The nature of study procedures may have contributed to the perceived and actual additional burden for study participants. While study activities relating to usual clinical procedures (*e.g.* trial of very early *versus* standard mobilisation post stroke; Bernhardt *et al*. 2008) may cause little additional

burden for study participants, studies of respiratory aspects, which are not routinely evaluated in acute stroke, can add considerable additional burden. Also, respiratory aspects of stroke impairment may be perceived as less obvious and therefore less relevant to the individual, which may impact on motivation to participate in this type of research. This may partly account for those patients who had no interest in the study (15% of eligible patients).

In the present study, a large proportion of eligible patients who decided not to take part did not provide a reason for declining. For ethical reasons, patients were not asked to give reasons for declining participation. Equally, participants who discontinued the study were not asked to provide a reason for discontinuing. However, understanding patients' motivations for (non-) participation and discontinuing gives valuable information for research design and management. Incorporating formalised procedures for exploring reasons for non-participation and discontinuation, for example through short qualitative interviews, may have provided further insights into barriers and facilitators of participant recruitment and retention, which could ultimately have been used to maximise recruitment and retention. This 'researching the research process' also ties in with initiatives for patient and public involvement in research, which have been shown to contribute to increased recruitment and improved trial designs (INVOLVE 2013, Brett *et al*. 2010, pp. 49-59, Staley 2009, pp. 30-49).

In the absence of formalised patient and public involvement in the present studies, sensitivity of the investigator towards barriers to research procedures identified two main facilitators to participant recruitment and retention: provision of transportation, and the option to conduct study visits at the participant's location. Mobility restrictions are a frequently mentioned problem in rehabilitation research, and adequate provisions are essential for the success of a study (Bell *et al*. 2008, Blanton *et al*. 2006). With no financial incentives for study participants, strategies to minimise the burden of travelling to and from research visits appeared to make a substantial difference to participants. Further, an appreciation of the local stroke service pathways and the investigator's flexibility around the clinical service (*i.e.* making use of clinical 'down time', such as weekday evenings and weekends) proved useful in maximising opportunities for study activities.

8.3 The present research in context

The present studies relate to four wider areas of research: respiratory function and impairment following stroke; strategies for reducing pneumonia incidence after stroke; strategies for improving neurologically impaired cough; and the application of RMT in stroke rehabilitation.

8.3.1 Respiratory function and impairment following stroke

There is comparatively little research into how stroke affects respiratory function, as opposed to the other possible sequelae of stroke. Historically, this may be due to the understanding that central neural control of automatic respiration is located at the level of the brainstem; and that the most dramatic potential effect of localised stroke on respiratory drive, *i.e.* acute respiratory failure, is seen in brainstem stroke and not in localised stroke lesions elsewhere in the brain (not including the effect of raised intracranial pressure and coning in severe malignant stroke) (Howard *et al*. 2001, Bogousslavsky *et al.* 1990, Patterson & Grabois 1986, Levin & Margolis 1977, Devereaux *et al*. 1973). Also, the human cardio-respiratory system adapts well to loss of respiratory capacity to a certain extent; and stroke survivors may not experience respiratory limitation, such as shortness of breath on exertion, within the often limited cardio-respiratory demands of post-stroke rehabilitation and the more sedentary lifestyle of many stroke survivors (English *et al*. 2014, Field *et al*. 2013). In this respect, respiratory limitations following stroke may not be as clinically evident as other stroke-related impairments, leading to lower awareness and priority for research. Nevertheless, there is some evidence available to demonstrate various aspects of respiratory impairment in stroke.

While insult to the brainstem respiratory centres can result in acute respiratory failure and death, a number of studies have reported more subtle disturbances in respiratory patterns, which have been described as Cheyne-Stokes or sleep-apnoea type respiration. These

breathing patterns have been observed in both sub- and supra-tentorial stroke, with no evident links to particular stroke lesion sites (Siccoli *et al*. 2008, Howard *et* al. 2001, Bassetti *et al*. 1997, Nachtman *et al*. 1995, Askenasy *et al*. 1988, Lee *et al*. 1971). Previously it was thought that these pathological respiratory patterns may be caused by stroke (Cherkassky *et al*. 2003). More recent evidence suggests that pre-existing obstructive sleep apnoea is an independent risk factor for stroke (Culebras 2014); and abnormal respiratory patterns detected post stroke may partly be accounted for by previously undiagnosed obstructive sleep apnoea.

An aspect of stroke-related respiratory impairment that has been investigated relatively frequently is diaphragm function. In several studies diaphragm excursions in stroke survivors were compared with healthy control subjects. Some authors found that hemi-diaphragm excursions on the contra-lesion side can be significantly reduced in severely impaired stroke patients (de Almeida *et al*. 2011, Lanini *et al*. 2003, Khedr *et al*. 2000, Cohen *et al*. 1994, Santamaria & Ruiz 1988, Fluck 1966, Korzcyn 1969, Smith 1962), while others reported bilaterally reduced diaphragm excursion following stroke regardless of lesion laterality (Houston *et al*. 1995), or minimal abnormality only (de Almeida *et al*. 2011, Freeman *et al*. 2006, Laghi and Tobin 2003, McMahon & Heyman 1974, Lee *et al*. 1971). More recent work (Voyvoda *et al*. 2012) identified varying patterns of hemi-diaphragm excursions after stroke, suggesting differences according to individual variation in neuromuscular pathways and stroke lesion sites. Reduced electro-myographic activity of the diaphragm and intercostal muscles on the stroke-affected side has also been reported (Przedborski *et al*. 1988, DeTroyer *et al*. 1981). Several studies have used trans-cranial magnetic stimulation to show the disruption stroke can cause to cortico-respiratory projections, with unelicitable or abnormal (longer latency, lower amplitude) motor evoked potentials compared to healthy control subjects (Harraf *et al*. 2008, Urban *et al*. 2002, Khedr *et al*. 2000, Similowski *et al*. 1996).

Volitional functional respiratory tests, such as spirometry, maximal mouth pressure measurements and maximal voluntary ventilation, have been conducted in stroke survivors in a number of studies (Pollock *et al*. 2012, Voyvoda *et al*. 2012, Zhou *et al*. 2012, de Almeida 2011, Jandt *et al*. 2011, Yoon *et al*. 2011, Ward *et al*. 2010, Harraff *et al*. 2008, Teixeira-Salmela *et al*.

2005, Lanini *et al*. 2003, Khedr *et al*. 2000, Nuzzo *et al*. 1999). Cough function following stroke has also been investigated in several studies, and these have been summarised in the introduction to this thesis (section 1.3.4). Overall, there is good evidence to show that stroke patients can exhibit considerable reductions in these aspects of respiratory function when compared with healthy control subjects. At the same time, there is wide variation in these parameters within the stroke population, which mirrors variations in severity of stroke impairment as well as natural variation observed in the healthy population.

In relation to this body of evidence, two points can be made from the findings of the present studies. First, with respect to exploring respiratory function and impairment after stroke, the present work adds the first description of cough frequency to the literature. Although these findings are derived from a small and selective convenience sample, it was possible to validate an automated cough frequency measurement system and capture longitudinal cough frequency data during the first three months following stroke. These data indicate some differences to normal cough frequency in humans and may serve hypothesis generation and further research. Second, with respect to the observed range in the various respiratory parameters measured, the data from the present studies mirror the variation seen in cross-sectional physiological studies. This may indicate that, in order to provide clinically useful information and lead to clinically meaningful intervention, assessments of respiratory function after stroke need to be conducted on individual basis and interpreted in the context of the individual patient. In this respect, respiratory impairment after stroke may require very much the same principal approach as stroke impairments of other body functions and systems, *i.e.* individualised assessment and intervention, as opposed to standardised treatments which are delivered routinely.

8.3.2 Reducing pneumonia incidence after stroke

Pneumonia following stroke presents a multi-factorial clinical picture. Accordingly, various avenues for reducing PSP incidence have been and are currently being pursued. An overview of different approaches has been given in the introduction to this thesis (section 1.2.4), and further to this three brief points shall be made here. First, compared to past and current studies,

the present research was novel in that it targeted cough effectiveness as a strategy to reduce PSP incidence. The research was based on a reasoned assumption, which was that stronger cough (*i.e.* higher expiratory cough flow) protects from aspiration-related pneumonia following stroke. This assumption had previously not been supported by data, likely because cough flow is not routinely captured in clinical practice and therefore not conveniently studied as a risk factor for PSP. However, there was some experimental evidence from a mechanical model (King *et al*. 1985). During the course of the present studies, it was possible to support this assumption through the retrospective analysis of cough flow as a predictor/mediator of PSP incidence (chapter 6); and while it would be desirable to replicate these findings in a dedicated study, these findings are original and add new information to the understanding of how cough mediates pneumonia risk after stroke. Second, it is noteworthy that the intention of the intervention was to achieve a restorative effect (as opposed to a supportive effect, such as is provided through manual thoraco-abdominal compression or the use of mechanical cough assist devices for the improvement of expiratory cough flow). And third, the intervention required active engagement from the participant, which contributed to defining the target population. It is acknowledged that this requirement for active engagement in the intervention largely defined and also narrowed the eligible patient pool. More severely affected individuals, who may present with fluctuating levels of consciousness and inability to follow instructions, are also among the higher risk groups for PSP. In future studies, passively administered treatments for the improvement of cough effectiveness may therefore be considered in order to allow inclusion of this more severely affected group of stroke survivors; whereby these treatments may exert a restorative or a supportive effect.

8.3.3 Improving neurologically impaired cough

Although various strategies for improving neurologically impaired cough have been researched in the past, the current evidence base is thin and there remains scope for further research and development (Jones *et al*. 2012, Boitano 2006). Among approaches to improving cough, supportive and restorative strategies may be distinguished. Supportive strategies aim to maximise cough effectiveness or secretion clearance by use of non-invasive cough-

augmentation techniques and devices. These techniques have mostly been investigated in populations with neurodegenerative conditions or spinal cord injury, to offer temporary support during acute episodes of respiratory care, or as long-term measures to prevent respiratory complications (Jones *et al*. 2012, Boitano 2006). Restorative strategies aim to achieve lasting improvement in cough function by delivering a training stimulus and achieving a training effect, which was the approach taken in the present research.

A supportive cough-augmentation technique that has long been in use clinically is manually assisted coughing. The patient's cough effort is supported by abdominal or thoracic compression performed by another person, resulting in higher peak cough flow (Boitano 2006, Kang *et al*. 2006a, Trebbia *et al*. 2005, Sivasothy *et al*. 2001, Braun *et al*. 1984, Kirby *et al*. 1966). Cough can also be augmented through hyperinflation manoeuvres. Passive increase in pre-cough inspiratory volume is achieved by manual hyperinflation or non-invasive ventilation devices such as intermittent positive pressure breathing (IPPB). The resulting increase in peak cough flow is attributed to both the increase in lung volume, providing more volume to be expelled, and elastic recoil energy tapped with increased lung and chest wall expansion (Boitano 2006, Kang *et al*. 2006a, Sivasothy *et al*. 2001, Bach 1993). A small number of studies investigated functional electrical and magnetic stimulation of the expiratory muscles for cough augmentation. These techniques appear viable, but require further research and development (Boitano 2006, Taylor *et al*. 2002, Lin *et al*. 1998, Jaeger *et al*. 1993). Lastly, mechanical in-exsufflation for cough augmentation was first described in the 1950s, but has recently gained support from a growing evidence base (Boitano 2006, Barach & Beck 1954, Beck & Barach 1954, Barach *et al.* 1953). This non-invasive device supports pre-cough inspiration through positive pressure, and the expulsive phase of cough through negative pressure (vacuum) applied to the airways. Several studies have demonstrated the effect of mechanical in-exsufflation on increasing peak cough flow (Chatwin *et al*. 2003, Mustafa *et al*. 2003, Bach 1993) and the clinical utility of the device (Vianello *et al*. 2005, Miske *et al*. 2004, Sancho *et al*. 2003, Marchant & Fox 2002, Garstang *et al*. 2000, Hanayama *et al*. 1997).

Restorative therapeutic modalities that have been investigated are respiratory muscle training and 'exercise' in the sense of whole body exercise or pulmonary rehabilitation-type exercise (Jones *et al*. 2012). In populations with neurodegenerative conditions these interventions have been researched to address respiratory problems in general; however, cough outcome parameters have rarely been measured. The small number of studies that included cough outcomes presents a mixed picture. Gosselink *et al*. (2000) investigated three months of expiratory muscle training in a randomised controlled trial in non-ambulatory multiple-sclerosis patients. Cough function was assessed using the Pulmonary Index, a composite score of objectively and subjectively rated cough effectiveness. This showed a small but statistically significant improvement in the intervention group compared to the control group, although it is difficult to judge the clinical relevance of this improvement. Chiara *et al*. (2006) studied the effect of eight weeks of expiratory muscle training in a cohort of patients with multiple sclerosis. Cough was assessed using cough flow measurements, and showed statistically significant improvement only in patients with moderate disease severity, but not with mild disease severity. Pitts *et al*. (2009) investigated four weeks of expiratory muscle training in a cohort of patients with Parkinson's disease. Cough flow measurements showed no statistically significant increase in PECF from baseline to post training; but glottis compression time and rise time to PECF reduced post training, which also resulted in a statistically significant increase in cough volume acceleration post training. The authors offer a discussion of the relative importance of PECF in comparison with other parameters that can be derived from a cough-flow trace (*i.e.* glottis compression time, rise time to PECF and cough volume acceleration); but at present it remains difficult to interpret how change in parameters other than PECF relates to cough effectiveness or protection from aspiration. Lastly, Kim *et al*. (2009, Kim & Sapienza 2005) investigated four weeks of expiratory muscle training in a group of healthy sedentary elderly subjects. Cough was evaluated through cough flow traces of capsaicin-induced reflex coughs. At the post-training time point, there was a statistically significant improvement in PECF as well as expiratory cough volume, and a significant reduction in glottis compression time.

Overall, these studies illustrate that research of restorative treatments for cough in clinical populations is in its beginning stages, with heterogeneity in study methodologies (in particular

regarding methods of assessing cough effectiveness) and interesting but overall inconclusive findings.

8.3.4 Respiratory muscle training in stroke rehabilitation

To date, most research of RMT has been conducted in healthy and athletic populations. Although the number of clinical studies is growing, the application of RMT in clinical practice remains an emerging area of investigation. As might be expected in such a case, investigators' approaches vary as the potential applications and benefits of RMT are explored, which is reflected in different study rationales and selection of outcome measures. Previous studies of RMT in stroke (section 1.5) and in other neurological conditions (Pollock *et al*. 2012) have generally focussed on physiological outcomes, whereby the most consistently included outcome parameters have been inspiratory and expiratory maximal mouth pressures. Various other physiological measures relating to lung function (*e.g.* spirometry) and cardio-respiratory endurance (*e.g.* peak oxygen consumption) have also been applied.

At the level of physiological impairment, controlled studies generally demonstrate some treatment effect of RMT. Arguably, improvement in physiological parameters alone does not warrant implementation of an investigational intervention, but there needs to be evidence of clinically meaningful patient benefit. For the application of RMT in stroke, this has not been convincingly established to date. In the two previous controlled trials of RMT in stroke with acceptable study quality (Britto *et al.* 2011, Sutbeyaz *et al.* 2010), those outcome measures that related directly to everyday life and general wellbeing, *i.e.* measures of activity, participation and quality of life, showed little treatment effect.

As for any clinical research, studies of RMT should be based on a considered rationale; and once patient safety and physiological effect have been demonstrated, the inclusion of clinically meaningful endpoints becomes increasingly relevant. To date, the accumulated evidence does indeed demonstrate that RMT is a generally safe, well tolerated and also inexpensive treatment

intervention in clinical populations. Because of that, RMT may lend itself to being researched or even prescribed in clinical practice 'because we can'. Acute and sub-acute stroke rehabilitation is time sensitive, and there is the need to focus treatment on the most time-effective and meaningful interventions. It may therefore be recommended that in the rationale for future studies of RMT in stroke the following points find particular consideration: How likely is it that the expected physiological effects of RMT will translate into meaningful patient benefit? And will the time spent on RMT be proportionate to other demands on the patient and the loss of opportunity to participate in other therapeutic activities? Most likely, these considerations will lead to different conclusions for the acute, sub-acute or chronic portion of the post-stroke pathway.

8.4 Suggestions for further studies

During the course of research presented in this thesis, potential areas for further studies have become apparent. These are given here in three topic summaries, which offer brief descriptions rather than fully developed specific research questions.

8.4.1 Methods of measuring cough effectiveness

The work investigating accuracy of devices for measuring peak cough flow (chapter 4) has shown that this area may warrant further research and development. The clinical usefulness of cough measurement could potentially be improved by re-examining current concepts of 'cough strength' and 'cough effectiveness'; and the methods by which these are measured. The measurement of peak cough flow clearly presents considerable challenges, and alternative measures of cough effectiveness could be explored, such as cough sound pressure level or mouth pressure during cough. The field may also benefit from a guideline or consensus statement on the measurement of cough effectiveness, to summarise current knowledge and provide guidance to clinicians and researchers. This work could best be addressed in an inter-disciplinary collaboration between physicists, respiratory physiologists and clinicians with an interest in cough effectiveness.

8.4.2 Features of cough following stroke

There is scope to further explore features of cough following stroke, both on a physiological level and in a clinical context. Physiological work could further investigate the role of glottis and intrinsic laryngeal muscle function. These may be more relevant for cough impairment after stroke than it was thought in the rationale for the present trial of RMT (section 1.3.4). Methods such as electro-myography or ultra-sonography may be able to give a better understanding of glottis function during cough, and findings may be useful in the design of novel treatment approaches for optimising cough after stroke.

The present studies have contributed somewhat towards the current understanding of the inter-relationship between swallowing impairment, pneumonia and cough after stroke. Stronger cough was shown to be protective from PSP in patients with unsafe swallow, but not in patients with safe swallow (chapter 6). Cough frequency after stroke was shown to be significantly higher than in healthy non-smokers, but there was no difference between patients with safe and unsafe swallow (chapter 7). These findings may warrant replicating in a dedicated observational study, which could also incorporate further measures of cough function (*e.g.* reflex cough sensitivity) and more detailed assessment of swallowing impairment (*i.e.* instrumental swallowing assessment). Such a study could add to the current understanding of how aspects of cough modify PSP risk after stroke, and contribute to the identification of targets for preventive therapies.

8.4.3 Strategies for improving cough effectiveness in acute stroke

Maximising cough effectiveness remains a viable target for PSP prevention strategies. The present trial showed that an intervention that requires active engagement from participants limits the eligible patient pool, which impacts on the feasibility of conducting this type of research. However, it may be warranted to explore treatment options which do not require

active patient engagement. The advantage of this could be twofold. First, patients with more severe stroke impairment who are inconsistent in following instructions could potentially be included for such a treatment. These patients are often also at higher risk of PSP, for example due to fluctuating levels of consciousness. Second, an intervention that is delivered passively is potentially less likely to detract from patients' capacity to participate in active rehabilitation activities, as it may draw less on patients' mental and physical resources.

Devising novel investigational treatment approaches provides an opportunity for innovation and may draw on previous research of cough-strengthening interventions as well as other work in the field of cough or stroke care. Suggestions may include: treatment modalities using electrical current stimulation of respiratory muscles; face masks with integrated valves for the delivery of added airway resistance; stimulated coughing using nebulised or aerosolised cough stimulants; or non-invasive stimulation of the central nervous system, such as TMS or trans-cranial direct current stimulation (tDCS).

References

ADAMSON, J., BESWICK, A. and EBRAHIM, S., 2004. Is stroke the most common cause of disability? *Journal of Stroke and Cerebrovascular Diseases,* **13**(4), pp. 171-177.

ADDINGTON, W.R., STEPHENS, R., WIDDICOMBE, J. and REKAB, K., 2005. Effect of stroke location on the laryngeal cough reflex and pneumonia risk. *Cough,* **1**(1), pp. 4.

ADDINGTON, W.R., STEPHENS, R.E. and GILLILAND, K.A., 1999b. Assessing the laryngeal cough reflex and the risk of developing pneumonia after stroke: An interhospital comparison. *Stroke,* **30**(6), pp. 1203-1207.

ADDINGTON, W.R., STEPHENS, R.E., GILLILAND, K. and RODRIGUEZ, M., 1999a. Assessing the laryngeal cough reflex and the risk of developing pneumonia after stroke. *Archives of Physical Medicine and Rehabilitation,* **80**(2), pp. 150-154.

AMERICAN THORACIC SOCIETY (ATS), 2004. Respiratory care of the patient with Duchenne muscular dystrophy. *American Journal of Respiratory and Critical Care Medicine,* **170**(4), pp. 456-465.

AMERICAN THORACIC SOCIETY (ATS), 1995. Standardization of spirometry, 1994 update. *American Journal of Respiratory and Critical Care Medicine,* **152**(3), pp. 1107-1136.

AMERICAN THORACIC SOCIETY (ATS) & EUROPEAN RESPIRATORY SOCIETY (ERS), 2002. ATS/ERS statement on respiratory muscle testing. *American Journal of Respiratory and Critical Care Medicine,* **166**(4), pp. 518-624.

ASKENASY, J.J. and GOLDHAMMER, I., 1988. Sleep apnea as a feature of bulbar stroke. *Stroke,* **19**(5), pp. 637-639.

ASLANYAN, S., WEIR, C.J., DIENER, H.-C., KASTE, M., LEES, K.R. and THE GAIN INTERNATIONAL STEERING COMMITTEE AND INVESTIGATORS, 2004. Pneumonia and urinary tract infection after acute ischaemic stroke: A tertiary analysis of the GAIN International trial. *European Journal of Neurology,* **11**(1), pp. 49-53.

BACH, J.R., 1993. Mechanical insufflation-exsufflation. Comparison of peak expiratory flows with manually assisted and unassisted coughing techniques. *CHEST Journal,* **104**(5), pp. 1553-1562.

BACH, J.R., GONCALVES, M.R., PAEZ, S., WINCK, J.C., LEITAO, S. and ABREU, P., 2006. Expiratory flow maneuvers in patients with neuromuscular diseases. *American Journal of Physical Medicine and Rehabilitation,* **85**(2), pp. 105-111.

BACH, J.R., ISHIKAWA, Y. and KIM, H., 1997. Prevention of pulmonary morbidity for patients with Duchenne muscular dystrophy. *CHEST Journal,* **112**(4), pp. 1024-1028.

BACH, J.R. and SAPORITO, L.R., 1996. Criteria for extubation and tracheostomy tube removal for patients with ventilatory failure: A different approach to weaning. *CHEST Journal,* **110**(6), pp. 1566-1571.

BACH, J.R., 1995. Amyotrophic lateral sclerosis: Predictors for prolongation of life by noninvasive respiratory aids. *Archives of Physical Medicine and Rehabilitation,* **76**(9), pp. 828-832.

BARACH, A.L., BECK, .G.J., 1954. Exsufflation with negative pressure: Physiologic and clinical studies in poliomyelitis, bronchial asthma, pulmonary emphysema, and bronchiectasis. *Archives of Internal Medicine,* **93**(6), pp. 825-841.

BARACH, A.L.M.D., BECK, G.J.M.D. and SMITH, W., 1953. Mechanical production of expiratory flow rates surpassing the capacity of human coughing. *American Journal of the Medical Sciences,* **226**(3), pp. 241-248.

BASSETTI, C., ALDRICH, M.S. and QUINT, D., 1997. Sleep-disordered breathing in patients with acute supra- and infratentorial strokes: A prospective study of 39 patients. *Stroke,* **28**(9), pp. 1765-1772.

BECK, G.J. and BARACH, A.L., 1954. Value of mechanical aids in the management of a patient with poliomyelitis. *Annals of Internal Medicine,* **40**(6), pp. 1081-1094.

BELL, K.R., HAMMOND, F., HART, T., BICKETT, A.K., TEMKIN, N.R. and DIKMEN, S., 2008. Participant recruitment and retention in rehabilitation research. *American Journal of Physical Medicine and Rehabilitation,* **87**(4), pp. 330-338.

BERNHARDT, J., DEWEY, H., THRIFT, A., COLLIER, J. and DONNAN, G., 2008. A very early rehabilitation trial for stroke (AVERT): Phase II safety and feasibility. *Stroke,* **39**(2), pp. 390-396.

BHATNAGAR, P., SCARBOROUGH, P., SMEETON, N. and ALLENDER, S., 2010. The incidence of all stroke and stroke subtype in the United Kingdom, 1985 to 2008: A systematic review. *BMC Public Health,* **10**(1), p. 539.

BIANCHI, A.L. and GESTREAU, C., 2009. The brainstem respiratory network: An overview of a half century of research. *Respiratory Physiology and Neurobiology,* **168**(1–2), pp. 4-12.

BIRRING, S.S., 2011. Controversies in the evaluation and management of chronic cough. *American Journal of Respiratory and Critical Care Medicine,* **183**(6), pp. 708-715.

BIRRING, S.S., FLEMING, T., MATOS, S., RAJ, A.A., EVANS, D.H. and PAVORD, I.D., 2008. The Leicester Cough Monitor: Preliminary validation of an automated cough detection system in chronic cough. *European Respiratory Journal,* **31**(5), pp. 1013-1018.

BIRRING, S.S., MATOS, S., PATEL, R.B., PRUDON, B., EVANS, D.H. and PAVORD, I.D., 2006. Cough frequency, cough sensitivity and health status in patients with chronic cough. *Respiratory Medicine,* **100**(6), pp. 1105-1109.

BLAND, J.M. and ALTMAN, D.G., 1999. Measuring agreement in method comparison studies. *Statistical methods in medical research,* **8**(2), pp. 135-160.

BLANTON, S., MORRIS, D.M., PRETTYMAN, M.G., MCCULLOCH, K., REDMOND, S., LIGHT, K.E. and WOLF, S.L., 2006. Lessons learned in participant recruitment and retention: The EXCITE trial. *Physical Therapy,* **86**(11), pp. 1520-1533.

BOGOUSSLAVSKY, J., KHURANA, R., DERUAZ, J.P., HORNUNG, J.P., REGLI, F., JANZER, R. and PERRET, C., 1990. Respiratory failure and unilateral caudal brainstem infarction. *Annals of Neurology,* **28**(5), pp. 668-673.

BOITANO, L.J., 2006. Management of airway clearance in neuromuscular disease. *Respiratory Care,* **51**(8), pp. 913-924.

BOTT, J., BLUMENTHAL, S., BUXTON, M., ELLUM, S., FALCONER, C., GARROD, R., HARVEY, A., HUGHES, T., LINCOLN, M., MIKELSONS, C., POTTER, C., PRYOR, J., RIMINGTON, L., SINFIELD, F., THOMPSON, C., VAUGHN, P. and WHITE, J., 2009. Guidelines for the physiotherapy management of the adult, medical, spontaneously breathing patient. *Thorax,* **64**(Suppl 1), pp. i1-i52.

BRAUN, J.S., PRASS, K., DIRNAGL, U., MEISEL, A. and MEISEL, C., 2007. Protection from brain damage and bacterial infection in murine stroke by the novel caspase-inhibitor Q-VD-OPH. *Experimental Neurology,* **206**(2), pp. 183-191.

BRAUN, S.R., GIOVANNONI, R. and O'CONNOR, M., 1984. Improving the cough in patients with spinal cord injury. *American Journal of Physical Medicine and Rehabilitation,* **63**(1), pp. 1-10.

BRETT, J., STANISZEWSKA, S., MOCKFORD, C., SEERS, K., HERRON-MARX, S. and BAYLISS, H., 2010. *The PIRICOM study: A systematic review of the conceptualisation, measurement, impact and outcomes of patients and public involvement in health and social care research.* London: United Kingdom Clinical Research Collaboration.

BRITO, M.F., MOREIRA, G.A., PRADELLA-HALLINAN, M. and TUFIK, S., 2009. Air stacking and chest compression increase peak cough flow in patients with Duchenne musclular dysptrophy. *Jornal Brasileiro de Pneumologia,* **35**, pp. 973-979.

BRITTO, R.R., REZENDE, N.R., MARINHO, K.C., TORRES, J.L., PARREIRA, V.F. and TEIXEIRA-SALMELA, L., 2011. Inspiratory muscular training in chronic stroke survivors: A randomized controlled trial. *Archives of Physical Medicine and Rehabilitation,* **92**(2), pp. 184-190.

BROOKS, S., 2011. Perspective on the human cough reflex. *Cough,* **7**(1), pp. 10.

BROWN M, G.M., 1973. Mortality factors in patients with acute stroke. *JAMA,* **224**(11), pp. 1493-1495.

CANNING, B.J., 2006. Anatomy and neurophysiology of the cough reflex : ACCP evidence-based clinical practice guidelines. *CHEST Journal,* **129**(Suppl1), pp. 33S-47S.

CANNING, B.J., MORI, N. and MAZZONE, S.B., 2006. Vagal afferent nerves regulating the cough reflex. *Respiratory Physiology and Neurobiology,* **152**(3), pp. 223-242.

CARDOSO, F., DE ABREU, L., RAIMUNDO, R., FAUSTINO, N., ARAUJO, S., VALENTI, V., SATO, M., MARTINS, S. and TORQUATO, J., 2012. Evaluation of peak cough flow in Brazilian healthy adults. *International Archives of Medicine,* **5**(1), pp. 25.

CHATWIN, M., ROSS, E., HART, N., NICKOL, A.H., POLKEY, M.I. and SIMONDS, A.K., 2003. Cough augmentation with mechanical insufflation/exsufflation in patients with neuromuscular weakness. *European Respiratory Journal,* **21**(3), pp. 502-508.

CHERKASSKY, T., OKSENBERG, A., FROOM, P. and RING, H., 2003. Sleep-related breathing disorders and rehabilitation outcome of stroke patients: A prospective study. *American Journal of Physical Medicine and Rehabilitation,* **82**(6), pp. 452-455.

CHIARA, T., MARTIN, A.D., DAVENPORT, P.W. and BOLSER, D.C., 2006. Expiratory muscle strength training in persons with multiple sclerosis having mild to moderate disability: Effect on maximal expiratory pressure, pulmonary function, and maximal voluntary cough. *Archives of Physical Medicine and Rehabilitation,* **87**(4), pp. 468-473.

CHOW, S.-C., SHAO, J. and WANG, H., 2008. *Sample size calculations in clinical research.* 2nd edn. Boca Raton: Chapman & Hall/CRC.

CHRISTENSEN, M.C., VALIENTE, R., SAMPAIO SILVA, G., LEE, W.C., DUTCHER, S., GUIMARES ROCHA, M.S. and MASSARO, A., 2009. Acute treatment costs of stroke in Brazil. *Neuroepidemiology,* **32**(2), pp. 142-149.

CHUA, K.S.G. and KONG, K., 1996. Functional outcome in brain stem stroke patients after rehabilitation. *Archives of Physical Medicine and* Rehabilitation, 77(2), pp. 194-197.

CHUMBLER, N.R., WILLIAMS, L.S., WELLS, C.K., LO, A.C., NADEAU, S., PEIXOTO, A.J., GORMAN, M., BOICE, J.L., CONCATO, J. and BRAVATA, D.M., 2010. Derivation and validation of a clinical system for predicting pneumonia in acute stroke. *Neuroepidemiology,* **34**(4), pp. 193-199.

CHUNG, K.F., 2006. Measurement of cough. *Respiratory Physiology and Neurobiology,* **152**(3), pp. 329-339.

CLEARY, S., MISIASZEK, J.E., KALRA, S., WHEELER, S. and JOHNSTON, W., 2013. The effects of lung volume recruitment on coughing and pulmonary function in patients with ALS. *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration,* **14**(2), pp. 111-115.

COHEN, E., MIER, A., HEYWOOD, P., MURPHY, K., BOULTBEE, J. and GUZ, A., 1994. Diaphragmatic movement in hemiplegic patients measured by ultrasonography. *Thorax,* **49**(9), pp. 890-895.

CRAMER, S.C., 2008. Repairing the human brain after stroke: I. Mechanisms of spontaneous recovery. *Annals of Neurology,* **63**(3), pp. 272-287.

CULEBRAS, A., 2014. Sleep apnea and stroke. *Current Neurology and Neuroscience Reports,* **15**(1), pp. 1-7.

DAFTARY, A.S., CRISANTI, M., KALRA, M., WONG, B. and AMIN, R., 2007. Effect of long-term steroids on cough efficiency and respiratory muscle strength in patients with Duchenne muscular dystrophy. *Pediatrics,* **119**(2), pp. e320-e324.

D'AGOSTINO, R., 2000. Debate: The slippery slope of surrogate outcomes. *Current Controlled Trials in Cardiovascular Medicine,* **1**(2), pp. 76-78.

DAY, S.J. and GRAHAM, D.F., 1989. Sample size and power for comparing two or more treatment groups in clinical trials. *British Medical Journal,* **299**, pp. 663-665.

DE ALMEIDA, I.C.L., CLEMENTINO, A.C.C.R., ROCHA, E.H.T., BRANDAO, D.C. and DORNELAS DE ANDRADE, A., 2011. Effects of hemiplegy on pulmonary function and diaphragmatic dome displacement. *Respiratory Physiology and Neurobiology,* **178**(2), pp. 196-201.

DE TROYER, A., ZEGERS DE BEYL, D. and THIRION, M., 1981. Function of the respiratory muscles in acute hemiplegia. *The American Review of Respiratory Disease,* **123**, pp. 631-632.

DEAN, E., 2002. Effects of positioning and mobilization. In: J.A. PRYOR and S.A. PRASAD, eds, *Physiotherapy for respiratory and cardiac problems. Adults and paediatrics.* 3rd edn. Edinburgh: Churchill Livingstone, pp. 143-159.

DECALMER, S., STOVOLD, R., HOUGHTON, L.A., PEARSON, J., WARD, C., KELSALL, A., JONES, H., MCGUINNESS, K., WOODCOCK, A. and SMITH, J.A., 2012. Chronic cough: relationship between microaspiration, gastroesophageal reflux, and cough frequency. *CHEST Journal,* **142**(4), pp. 958-964.

DEMOULE, A., VERIN, E., MONTCEL, S.T.D. and SIMILOWSKI, T., 2008. Short-term training-dependent plasticity of the corticospinal diaphragm control in normal humans. *Respiratory Physiology and Neurobiology,* **160**(2), pp. 172-180.

DEROUESNE, C., CAMBON, H., YELNIK, A., DUYCKAERTS, C. and HAUW, J.J., 1993. Infarcts in the middle cerebral artery territory. *Acta Neurologica Scandinavica,* **87**(5), pp. 361-366.

DESPOPOULOS, A. and SILBERNAGEL, S., 2003. *Color atlas of physiology.* 5 edn. Stuttgart: Thieme.

DEVEREAUX, M.W., KEANE, J.R., DAVIS, R.L., 1973. Automatic respiratory failure: Report of two cases with pathologic study of one. *Archives of Neurology,* **29**(1), pp. 46-52.

DOHNA-SCHWAKE, C., RAGETTE, R., TESCHLER, H., VOIT, T. and MELLIES, U., 2006. IPPB-assisted coughing in neuromuscular disorders. *Pediatric Pulmonology,* **41**(6), pp. 551-557.

DOMHOLDT, E., 2005. *Rehabilitation research. Principles and applications.* 3rd edn. St Louis, MO: Elsevier Saunders.

DONNAN, G.A., FISHER, M., MACLEOD, M. and DAVIS, S.M., 2008. Stroke. *The Lancet,* **371**(9624), pp. 1612-1623.

DZIEWAS, R., RITTER, M., SCHILLING, M., KONRAD, C., OELENBERG, S., NABAVI, D.G., STÖGBAUER, F., RINGELSTEIN, E.B. and LÜDEMANN, P., 2004. Pneumonia in acute stroke patients fed by nasogastric tube. *Journal of Neurology, Neurosurgery and Psychiatry,* **75**(6), pp. 852-856.

ENGLISH, C., MANNS, P.J., TUCAK, C. and BERNHARDT, J., 2014. Physical activity and sedentary behaviors in people with stroke living in the community: A systematic review. *Physical Therapy,* **94**(2), pp. 185-196.

FERNANDES, F., MARTINS, S.R.G. and BONVENT, J.J., 2007. Efeito do treinamento muscular respiratorio por meio do manovacuometro e do threshold pep em pacientes hemipareticos hospitalizados. In: C. MUELLER-KARGER, S. WONG and A. CRUZ, eds, *IFMBE proceedings.* Springer Berlin Heidelberg, pp. 1199-1202.

FIELD, M.J., GEBRUERS, N., SHANMUGA SUNDARAM, T., NICHOLSON, S. and MEAD, G., 2013. Physical activity after stroke: A systematic review and meta-analysis. *ISRN Stroke*, 2013, pp. 13.

FINLAYSON, O., KAPRAL, M., HALL, R., ASLLANI, E., SELCHEN, D. and SAPOSNIK, G., 2011. Risk factors, inpatient care, and outcomes of pneumonia after ischemic stroke. *Neurology,* **77**(14), pp. 1338-1345.

FINUCANE, K.E., EGAN, B.A. and DAWSON, S.V., 1972. Linearity and frequency response of pneumotachographs. *Journal of Applied Physiology,* **32**(1), pp. 121-126.

FIORE, J.F., CHIAVEGATO, L.D., DENEHY, L., PAISANI, D.M. and FARESIN, S.M., 2008. Do directed cough maneuvers improve cough effectiveness in the early period after open heart surgery? Effect of thoracic support and maximal inspiration on cough peak expiratory flow, cough expiratory volume, and thoracic pain. *Respiratory Care,* **53**(8), pp. 1027-1034.

FLUCK, D.C., 1966. Chest movements in hemiplegia. *Clinical Science,* **31**, pp. 383-388.

FONTANA, G.A. and LAVORINI, F., 2006. Cough motor mechanisms. *Respiratory Physiology and Neurobiology,* **152**(3), pp. 266-281.

FREEMAN, W.D., SEN, S., ROY, T.K. and WIJDICKS, E.M., 2006. Cluster breathing associated with bihemispheric infarction and sparing of the brainstem. *Archives of Neurology,* **63**(10), pp. 1487-1490.

FREITAS, F.S., IBIAPINA, C.C., ALVIM, C.G., BRITTO, R.R. and PARREIRA, V.F., 2010. Relationship between cough strength and functional level in elderly. *Brazilian Journal of Physical Therapy,* **14**(6), pp. 470-476.

GARNER, J.S., JARVIS, W.R., EMORI, T.G., HORAN, T.C. and HUGHES, J.M., 1988. CDC definitions for nosocomial infections, 1988. *American Journal of Infection Control,* **16**(3), pp. 128-140.

GARSTANG, S.V., KIRSHBLUM, S.C. and WOOD, K.E., 2000. Patient preference for in-exsufflation for secretion management with spinal cord injury. *Journal of Spinal Cord Medicine,* **23**(2), pp. 80-85.

GAULD, L.M. and BOYNTON, A., 2005. Relationship between peak cough flow and spirometry in Duchenne muscular dystrophy. *Pediatric Pulmonology,* **39**(5), pp. 457-460.

GEDDES, E.L., O'BRIEN, K., REID, W.D., BROOKS, D. and CROWE, J., 2008. Inspiratory muscle training in adults with chronic obstructive pulmonary disease: An update of a systematic review. *Respiratory* Medicine, **102**(12), pp. 1715-1729.

GLADMAN, J.R.F., LINCOLN, N.B. and ADAMS, S.A., 1993. Use of the extended ADL Scale with stroke patients. *Age and Ageing,* **22**(6), pp. 419-424.

GOLDSTEIN, R.S., 1993. Pulmonary rehabilitation in chronic respiratory insufficiency. 3. Ventilatory muscle training. *Thorax,* **48**(10), pp. 1025-1033.

GOLLEE, H., HUNT, K.J., FRASER, M.H. and MCLEAN, A.N., 2008. Abdominal stimulation for respiratory support in tetraplegia: A tutorial review. *Journal of Automatic* Control, 18(2), pp. 85-92.

GORDON, C., HEWER, R.L. and WADE, D.T., 1987. Dysphagia in acute stroke. *British Medical Journal,* **295**(6595), pp. 411-414.

GOSNEY, M., MARTIN, M.V. and WRIGHT, A.E., 2006. The role of selective decontamination of the digestive tract in acute stroke. *Age and Ageing,* **35**(1), pp. 42-47.

GOSSELINK, R., DE VOS, J., VAN DEN HEUVEL, S.P., SEGERS, J., DECRAMER, M. and KWAKKEL, G., 2011. Impact of inspiratory muscle training in patients with COPD: What is the evidence? *European Respiratory Journal,* **37**(2), pp. 416-425.

GOSSELINK, R., KOVACS, L., KETELAER, P., CARTON, H. and DECRAMER, M., 2000. Respiratory muscle weakness and respiratory muscle training in severely disabled multiple sclerosis patients. *Archives of Physical Medicine and Rehabilitation,* **81**(6), pp. 747-751.

GOTTLIEB, D., KIPNIS, M., SISTER, E., VARDI, Y. and BRILL, S., 1996. Validation of the 50 ml^3 drinking test for evaluation of post-stroke dysphagia. *Disability and Rehabilitation,* **18**(10), pp. 529-532.

GOETZSCHE, P.C., LIBERATI, A., TORRI, V. and ROSSETTI, L., 1996. Beware of surrogate outcome measures. *International Journal of Technology Assessment in Health Care,* **12**(02), pp. 238-246.

CUESY, P.G., SOTOMAYOR, P.L. and PINA, J.O.T., 2010. Reduction in the incidence of poststroke nosocomial pneumonia by using the “Turn-mob” program. *Journal of Stroke and Cerebrovascular Diseases,* **19**(1), pp. 23-28.

GREAT BRITAIN. DEPARTMENT OF HEALTH, 2007. *National Stroke Strategy.* London: Department of Health.

HADJIKOUTIS, S., ECCLES, R. and WILES, C.M., 2000. Coughing and choking in motor neuron disease. *Journal of Neurology, Neurosurgery, and Psychiatry,* **68**(5), pp. 601-604.

HANAYAMA, K., ISHIKAWA, Y. and BACH, J.R., 1997. Amyothrophic lateral sclerosis: Successful treatment of mucous plugging by mechanical insufflation-exsufflation. *American Journal of Physical Medicine and Rehabilitation,* **76**(4),.

HANKINSON, J., STOCKS, J. and PESLIN, R., 1998. Reproducibility of lung volume measurements. *European Respiratory Journal,* **11**(3), pp. 787-790.

HANNAWI, Y., HANNAWI, B., RAO, C.P.V., SUAREZ, J.I. and BERSHAD, E.M., 2013. Stroke-associated pneumonia: Major advances and obstacles. *Cerebrovascular Diseases,* **35**(5), pp. 430-443.

HARRAF, F., WARD, K., MAN, W., RAFFERTY, G., MILLS, K., POLKEY, M., MOXHAM, J. and KALRA, L., 2008. Transcranial magnetic stimulation study of expiratory muscle weakness in acute ischemic stroke. *Neurology,* **71**(24), pp. 2000-2007.

HENON, H., GODEFROY, O., LEYS, D., MOUNIER-VEHIER, F., LUCAS, C., RONDEPIERRE, P., DUHAMEL, A. and PRUVO, J.P., 1995. Early predictors of death and disability after acute cerebral ischemic event. *Stroke,* **26**(3), pp. 392-398.

HEUSCHMANN, P.U., KOLOMINSKY-RABAS, P.L., MISSELWITZ, B., HERMANEK, P., LEFFMANN, C., JANZEN, R.W.C., ROTHER, J., BUECKER-NOTT, H.-J. and BERGER, K., 2004. Predictors of in-hospital mortality and attributable risks of death after ischemic stroke: The German stroke registers study group. *Archives of Internal Medicine,* **164**(16), pp. 1761-1768.

HILKER, R., POETTER, C., FINDEISEN, N., SOBESKY, J., JACOBS, A., NEVELING, M. and HEISS, W., 2003. Nosocomial pneumonia after acute stroke: Implications for neurological intensive care medicine. *Stroke,* **34**(4), pp. 975-981.

HINCHEY, J.A., SHEPHARD, T., FURIE, K., SMITH, D., WANG, D., and TONN, S., 2005. Formal dysphagia screening protocols prevent pneumonia. *Stroke,* **36**(9), pp. 1972-1976.

HOFFMANN, S., MALZAHN, U., HARMS, H., KOENNECKE, H., BERGER, K., KALIC, M., WALTER, G., MEISEL, A. and HEUSCHMANN, P.U., 2012. Development of a clinical score (A2DS2) to predict pneumonia in acute ischemic stroke. *Stroke,* **43**(10), pp. 2617-2623.

HOLAS, M.A., DEPIPPO, K.L. and REDING, M.J., 1994. Aspiration and relative risk of medical complications following stroke. *Archives of Neurology,* **51**(10), pp. 1051-1053.

HONG, K., KANG, D., KOO, J., YU, K., HAN, M., CHO, Y., PARK, J., BAE, H. and LEE, B., 2008. Impact of neurological and medical complications on 3-month outcomes in acute ischaemic stroke. *European Journal of Neurology,* **15**(12), pp. 1324-1331.

HOUSTON, J.G., MORRIS, A.D., GROSSET, D.G., LEES, K.R., MCMILLAN, N. and BONE, I., 1995. Ultrasonic evaluation of movement of the diaphragm after acute cerebral infarction. *Journal of Neurology, Neurosurgery and Psychiatry,* **58**(6), pp. 738-741.

HOWARD, R.S., RUDD, A.G., WOLFE, C.D. and WILLIAMS, A.J., 2001. Pathophysiological and clinical aspects of breathing after stroke. *Postgraduate Medical Journal,* **77**(913), pp. 700-702.

HULL, J., ANIAPRAVAN, R., CHAN, E., CHATWIN, M., FORTON, J., GALLAGHER, J., GIBSON, N., GORDON, J., HUGHES, I., MCCULLOCH, R., RUSSELL, R.R. and SIMONDS, A., 2012. British Thoracic Society guideline for respiratory management of children with neuromuscular weakness. *Thorax,* **67**(Suppl 1), pp. i1-i40.

HULZEBOS ERIK, H.J., SMIT, Y., HELDERS PAUL, P.J.M. and VAN MEETEREN NICO, L.U., 2012. Preoperative physical therapy for elective cardiac surgery patients. *Cochrane Database of Systematic* Reviews, (11), Art.No.:CD010118.

ICKENSTEIN, G.W., RIECKER, A., HAEHLIG, C., MUELLER, R., BECKER, U., REICHMANN, H. and PROSIEGEL, M., 2010. Pneumonia and in-hospital mortality in the context of neurogenic oropharyngeal dysphagia (NOD) in stroke and a new NOD step-wise concept. *Journal of Neurology,* **257**(9), pp. 1492-1499.

INDREDAVIK, B., ROHWEDER, G., NAALSUND, E. and LYDERSEN, S., 2008. Medical complications in a comprehensive stroke unit and an early supported discharge service. *Stroke,* **39**(2), pp. 414-420.

INTERCOLLEGIATE STROKE WORKING PARTY, 2012. *National clinical guideline for stroke.* 4th edn. London: Royal College of Physicians.

INTERCOLLEGIATE STROKE WORKING PARTY, 2011. *National sentinel stroke clinical audit 2010. Public report for England, Wales and Northern Ireland.* London: Royal College of Physicians.

INVOLVE, 2013. *Exploring the impact of public involvement on the quality of research: examples.* Eastleigh: INVOLVE.

IRWIN, R.S., BAUMANN, M.H., BOLSER, D.C., BOULET, L., BRAMAN, S.S., BRIGHTLING, C.E., BROWN, K.K., CANNING, B.J., CHANG, A.B., DICPINIGAITIS, P.V., ECCLES, R., GLOMB, W.B., GOLDSTEIN, L.B., GRAHAM, L.M., HARGREAVE, F.E., KVALE, P.A., LEWIS, S.Z., MCCOOL, F.D., MCCRORY, D.C., PRAKASH, U.B.S., PRATTER, M.R., ROSEN, M.J., SCHULMAN, E., SHANNON, J.J., SMITH HAMMOND, C. and TARLO, S.M., 2006. Diagnosis and management of cough: ACCP evidence-based clinical practice guidelines. *CHEST Journal,* **129**(Suppl1), pp. 1S-23S.

JACKSON, A.C. and VINEGAR, A., 1979. A technique for measuring frequency response of pressure, volume, and flow transducers. *Journal of Applied Physiology,* **47**(2), pp. 462-467.

JAEGER, R.J., TURBA, R.M., YARKONY, G.M. and ROTH, E.J., 1993. Cough in spinal cord injured patients: Comparison of three methods to produce cough. *Archives of Physical Medicine and Rehabilitation,* **74**(12), pp. 1358-1361.

JANDT, S.R., DA SIL CABALLERO, R.M., JUNIOR, L.A.F. and DIAS, A.S., 2011. Correlation between trunk control, respiratory muscle strength and spirometry in patients with stroke: An observational study. *Physiotherapy Research International,* **16**(4), pp. 218-224.

JENKINS, S. and TUCKER, B., 2002. Patients' problems, management and outcomes. In: J.A. PRYOR and S.A. PRASAD, eds, *Physiotherapy for respiratory and cardiac problems. Adults and paediatrics.* 3rd edn. Edinburgh: Churchill Livingstone, pp. 243-280.

JOFFE, M.M. and GREENE, T., 2009. Related causal frameworks for surrogate outcomes. *Biometrics,* **65**(2), pp. 530-538.

JOHNSON, E.R., MCKENZIE, S.W. and SIEVERS, A., 1993. Aspiration pneumonia in stroke. *Archives of Physical Medicine and Rehabilitation,* **74**, pp. 973-976.

JONES, U., ENRIGHT, S. and BUSSE, M., 2012. Management of respiratory problems in people with neurodegenerative conditions: A narrative review. *Physiotherapy,* **98**(1), pp. 1-12.

JUNG, J., SHIM, J., KWON, H., KIM, H. and KIM, B., 2014. Effects of abdominal stimulation during inspiratory muscle training on respiratory function of chronic stroke patients. *Journal of Physical Therapy Science,* **26**(1), pp. 73-76.

KALRA, L., 2010. Stroke rehabilitation 2009: Old chestnuts and new insights. *Stroke,* **41**(2), pp. e88-e90.

KALRA, L., YU, G., WILSON, K. and ROOTS, P., 1995. Medical complications during stroke rehabilitation. *Stroke,* **26**(6), pp. 990-994.

KANG, J., PARK, R., LEE, S., KIM, J., YOON, S. and JUNG, K., 2012. The effect of bedside exercise program on stroke patients with dysphagia. *Annals of Rehabilitation Medicine,* **36**(4), pp. 512-520.

KANG, S.W., SHIN, J.C., PARK, C.I., MOON, J.H., RHA, D.W. and CHO, D.-H., 2006b. Relationship between inspiratory muscle strength and cough capacity in cervical spinal cord injured patients. *Spinal Cord,* **44**(4), pp. 242-248.

KANG, S., KANG, Y., SOHN, H., PARK, J. and MOON, J., 2006a. Respiratory muscle strength and cough capacity in patients with Duchenne muscular dystrophy. *Yonsei Medical Journal,* **47**(2), pp. 184-190.

KATZAN, I.L., CEBUL, R.D., HUSAK, S.H., DAWSON, N.V. and BAKER, D.W., 2003. The effect of pneumonia on mortality among patients hospitalized for acute stroke. *Neurology,* **60**(4), pp. 620-625.

KATZAN, I.L., DAWSON, N.V., THOMAS, C.L., VOTRUBA, M.E. and CEBUL, R.D., 2007. The cost of pneumonia after acute stroke. *Neurology,* **68**(22), pp. 1938-1943.

KHEDR, E.M., EL SHINAWY, O., KHEDR, T., ABDEL AZIZ ALI, Y. and AWAD, E.M., 2000. Assessment of corticodiaphragmatic pathway and pulmonary function in acute ischemic stroke patients. *European Journal of Neurology,* **7**(5), pp. 509-516.

KIDD, D., LAWSON, J., NESBITT, R. and MACMAHON, J., 1995. The natural history and clinical consequences of aspiration in acute stroke. *Quarterly Journal of Medicine,* **88**(6), pp. 409-413.

KIM, J. and SAPIENZA, C.M., 2005. Implications of expiratory muscle strength training for rehabilitation of the elderly. Tutorial. *Journal of Rehabilitation Research and Development,* **42**(2), pp. 211-224.

KIM, J., DAVENPORT, P. and SAPIENZA, C., 2009. Effect of expiratory muscle strength training on elderly cough function. *Archives of Gerontology and* Geriatrics, 48(3), pp. 361-366.

KIM, K., FELL, D.W. and LEE, J.H., 2011. Feedback respiratory training to enhance chest expansion and pulmonary function in chronic stroke: A double-blind, randomized controlled study. *Journal of Physical Therapy Science,* **23**(1), pp. 75-79.

KIMURA, Y., TAKAHASHI, M., WADA, F. and HACHISUKA, K., 2013. Differences in the peak cough flow among stroke patients with and without dysphagia. *Journal of UOEH,* **35**(1), pp. 9-16.

KING, M., BROCK, G. and LUNDELL, C., 1985. Clearance of mucus by simulated cough. *Journal of Applied Physiology,* **58**(6), pp. 1776-1782.

KIRBY, N.A., BARNERIAS, M.J. and SIEBENS, A.A., 1966. An evaluation of assisted cough in quadriparetic patients. *Archives of Physical Medicine and Rehabilitation,* **47**(11), pp. 705-710.

KISHORE, A.K., VAIL, A., CHAMORRO, A., GARAU, J., HOPKINS, S.J., DI NAPOLI, M., KALRA, L., LANGHORNE, P., MONTANER, J., ROFFE, C., RUDD, A.G., TYRRELL, P.J., VAN DE BEEK, D., WOODHEAD, M., MEISEL, A. and SMITH, C.J., 2015. How is pneumonia diagnosed in clinical stroke research? A systematic review and meta-analysis. *Stroke*, **46**(5), pp. 1202-1209.

KOENNECKE, H.-C., BELZ, W., BERFELDE, D., ENDRES, M., FITZEK, S., HAMILTON, F., KREITSCH, P., MACKERT, B.-M., NABAVI, D.G., NOLTE, C.H., PÖHLS, W., SCHMEHL, I., SCHMITZ, B., VON BREVERN, M., WALTER, G. and HEUSCHMANN, P.U., 2011. Factors influencing in-hospital mortality and morbidity in patients treated on a stroke unit. *Neurology,* **77**(10), pp. 965-972.

KORCZYN, A.D., LEIBOWITZ, U. and BRUDERMAN, I., 1969. Involvement of the diaphragm in hemiplegia. *Neurology,* **19**, pp. 97-100.

LAGHI, F. and TOBIN, M.J., 2003. Disorders of the respiratory muscles. *American Journal of Respiratory and Critical Care Medicine,* **168**(1), pp. 10-48.

LAKSHMINARAYAN, K., TSAI, A.W., TONG, X., VAZQUEZ, G., PEACOCK, J.M., GEORGE, M.G., LUEPKER, R.V. and ANDERSON, D.C., 2010. Utility of dysphagia screening results in predicting poststroke pneumonia. *Stroke,* **41**(12), pp. 2849-2854.

LANGHORNE, P., STOTT, D.J., ROBERTSON, L., MACDONALD, J., JONES, L., MCALPINE, C., DICK, F., TAYLOR, G.S. and MURRAY, G., 2000. Medical complications after stroke: A multicenter study. *Stroke,* **31**(6), pp. 1223-1229.

LANINI, B., BIANCHI, R., ROMAGNOLI, I., COLI, C., BINAZZI, B., GIGLIOTTI, F., PIZZI, A., GRIPPO, A. and SCANO, G., 2003. Chest wall kinematics in patients with hemiplegia. *American Journal of Respiratory and Critical Care Medicine,* **168**(1), pp. 109-113.

LEE, J.H., SEO, K.C. and KIM, K., 2012. Measurement of changes in chest mobility and pulmonary functions in relation to stroke patients' positions. *Journal of Physical Therapy Science,* **24**(3), pp. 253-256.

LEE, M.C., KLASSEN, A.C. and RESCH, J.A., 1971. Respiratory pattern disturbances in ischemic cerebral vascular disease. *Stroke,* **5**(5), pp. 612-616.

LEE, S., SHAFE, A.C.E. and COWIE, M.R., 2011. UK stroke incidence, mortality and cardiovascular risk management 1999–2008: Time-trend analysis from the General Practice Research Database. *BMJ Open*, **1**(2), pp. e000269.

LEE, S.C., KANG, S., KIM, M.T., KIM, Y.K., CHANG, W.H. and IM, S.H., 2013. Correlation between voluntary cough and laryngeal cough reflex flows in patients with traumatic brain injury. *Archives of Physical Medicine and Rehabilitation*, 94(8), pp. 1580-1583.

LEINER, G.C.M.D., ABRAMOWITZ, S., SMALL, M.J.M.D. and STENBY, V.B., 1966. Cough peak flow rate. *American Journal of the Medical Sciences,* **251**(2), pp. 211-214.

LEITH, D.E. and BRADLEY, M., 1976. Ventilatory muscle strength and endurance training. *Journal of Applied Physiology,* **41**(4), pp. 508-516.

LEVIN, B.E. and MARGOLIS, G., 1977. Acute failure of automatic respirations secondary to a unilateral brainstem infarct. *Annals of Neurology,* **1**(6), pp. 583-586.

LIM, S.H.B., LIEU, P.K., PHUA, S.Y., SESHADRI, R., VENKETASUBRAMANIAN, N., LEE, S.H. and CHOO, P.W.J., 2001. Accuracy of bedside clinical methods compared with fiberoptic endoscopic examination of swallowing (FEES) in determining the risk of aspiration in acute stroke patients. *Dysphagia,* **16**(1), pp. 1-6.

LIN, S.-J., MCELFRESH, J., HALL, B., BLOOM, R. and FARRELL, K., 2012. Inspiratory muscle training in patients with heart failure: A systematic review. *Cardiopulmonary Physical Therapy Journal,* **23**(3), pp. 29-36.

LIN, V.W., HSIEH, C., HSIAO, I.N. and CANFIELD, J., 1998. Functional magnetic stimulation of expiratory muscles: a noninvasive and new method for restoring cough. *Journal of Applied Physiology,* **84**(4), pp. 1144-1150.

LLOYD, G., DEAN, C.M. and ADA, L., 2010. Issues in recruiting community-dwelling stroke survivors to clinical trials: The AMBULATE trial. *Contemporary Clinical Trials,* **31**(4), pp. 289-292.

LOMAURO, A., ROMEI, M., D'ANGELO, M.G. and ALIVERTI, A., 2014. Determinants of cough efficiency in Duchenne muscular dystrophy. *Pediatric Pulmonology,* **49**(4), pp. 357-365.

LOUDON, R.G. and SHAW, G.B., 1967. Mechanics of cough in normal subjects and in patients with obstructive respiratory disease. *American Review of Respiratory Diseases,* **96**(4), pp. 666-677.

MANN, G., HANKEY, G.J. and CAMERON, D., 1999. Swallowing function after stroke: Prognosis and prognostic factors at 6 months. *Stroke,* **30**(4), pp. 744-748.

MARCHANT, W.A. and FOX, R., 2002. Postoperative use of a cough-assist device in avoiding prolonged intubation. *British Journal of Anaesthesia,* **89**(4), pp. 644-647.

MARTINI, F.H., 2004. *Fundamentals of Anatomy & Physiology.* 6th edn. San Francisco: Benjamin Cummings.

MARTINO, R., FOLEY, N., BHOGAL, S., DIAMANT, N., SPEECHLEY, M. and TEASELL, R., 2005. Dysphagia after stroke: Incidence, diagnosis, and pulmonary complications. *Stroke,* **36**(12), pp. 2756-2763.

MASIERO, S., PIEROBON, R., PREVIATO, C. and GOMIERO, E., 2008. Pneumonia in stroke patients with oropharyngeal dysphagia: a six-month follow-up study. *Neurological Sciences,* **29**(3), pp. 139-145.

MATOS, S., BIRRING, S.S., PAVORD, I.D. and EVANS, D.H., 2007. An automated system for 24-h monitoring of cough frequency: the Leicester Cough Monitor. *IEEE Transactions on Bio-Medical Engineering,* **54**(8), pp. 1472-1479.

MATOS, S., BIRRING, S.S., PAVORD, I.D. and EVANS, D.H., 2006. Detection of cough signals in continuous audio recordings using hidden Markov models. *IEEE Transactions on Bio-Medical Engineering,* **53**(6), pp. 1078-1083.

MAZZONE, S., 2005. An overview of the sensory receptors regulating cough. *Cough,* **1**(1), pp. 2.

MCCONNELL, A.K. and ROMER, L.M., 2004. Respiratory muscle training in healthy humans: Resolving the controversy. *International Journal of Sports Medicine,* **25**(4), pp. 284-293.

MCCONNELL, A., 2013. *Respiratory muscle training. Theory and practice.* Edinburgh: Churchill Livingstone Elsevier.

MCCONNELL, A., 2011. *Breathe strong, perform better.* Leeds: Human Kinetics.

MCCONNELL, A.K. and GRIFFITHS, L.A., 2010. Acute cardiorespiratory responses to inspiratory pressure threshold loading. *Medicine and Science in Sports and Exercise,* **42**(9), pp. 1696-1703.

MCMAHON, S.M. and HEYMAN, A., 1974. The mechanics of breathing and stabilization of ventilation in patients with unilateral cerebral infarction. *Stroke,* **5**(4), pp. 518-527.

MEHTA, C.R. and PATEL, N.R., 1995. Exact logistic regression: theory and examples. *Statistics in Medicine,* **14**, pp. 2143-2160.

MILLER, M.R., ATKINS, P.R. and PEDERSEN, O.F., 2003. Inadequate peak expiratory flow meter characteristics detected by a computerised explosive decompression device. *Thorax,* **58**(5), pp. 411-416.

MILLER, M.R., HANKINSON, J., BRUSASCO, V., BURGOS, F., CASABURI, R., COATES, A., CRAPO, R., ENRIGHT, P., VAN DER GRINTEN, C.P.M., GUSTAFSSON, P., JENSEN, R., JOHNSON, D.C., MACINTYRE, N., MCKAY, R., NAVAJAS, D., PEDERSEN, O.F., PELLEGRINO, R., VIEGI, G. and WANGER, J., 2005. Standardisation of spirometry. *European Respiratory Journal*, **26**(2), pp. 319-338.

MILLER, M.R., LLOYD, J. and BRIGHT, P., 2002. Recording flow in the first second of a maximal forced expiratory manoeuvre: influence of frequency content. *European Respiratory Journal,* **19**(3), pp. 530-533.

MILLER, R.M. and BRITTON, D., 2011. *Dysphagia in neuromuscular diseases.* San Diego: Plural Publishing Inc.

MISKE, L.J., HICKEY, E.M., KOLB, S.M., WEINER, D.J. and PANITCH, H.B., 2004. USe of the mechanical in-exsufflator in pediatric patients with neuromuscular disease and impaired cough. *CHEST Journal,* **125**(4), pp. 1406-1412.

MORICE, A.H., FONTANA, G.A., BELVISI, M.G., BIRRING, S.S., CHUNG, K.F., DICPINIGAITIS, P.V., KASTELIK, J.A., MCGARVEY, L.P., SMITH, J.A., TATAR, M. and WIDDICOMBE, J., 2007. ERS guidelines on the assessment of cough. *European Respiratory Journal,* **29**(6), pp. 1256-1276.

MUSTFA, N., AIELLO, M., LYALL, R.A., NIKOLETOU, D., OLIVIERI, D., LEIGH, P.N., DAVIDSON, A.C., POLKEY, M.I. and MOXHAM, J., 2003. Cough augmentation in amyotrophic lateral sclerosis. *Neurology,* **61**(9), pp. 1285-1287.

NACHTMANN, A., SIEBLER, M., ROSE, G., SITZER, M. and STEINMETZ, H., 1995. Cheyne-Stokes respiration in ischemic stroke. *Neurology,* **45**(4), pp. 820-821.

NATIONAL AUDIT OFFICE, 2010. *Progress in improving stroke care.* London: The Stationary Office.

NATIONAL COLLABORATING CENTRE FOR CHRONIC CONDITIONS, 2008. *Stroke: national clinical guideline for diagnosis and initial management of acute stroke and transient ischaemic attack (TIA).* London: Royal College of Physicians.

NHS CAREERS, no date, Agenda for change - pay rates. Available at: http://www.nhscareers.nhs.uk/working-in-the-nhs/pay-and-benefits/agenda-for-change-pay-rates/ [Accessed on: 30 May 2014].

NUZZO, N.A., BRONSON, L.A., MCCARTHY, T. and MASSERY, M., 1999. Respiratory muscle strength and endurance following a CVA. *Neurology Report,* **23**(1), pp. 25-27.

OPPENHEIMER, S. and HACHINSKI, V., 1992. Complications of acute stroke. *The Lancet*, **339**(8795), pp. 721-724.

OVBIAGELE, B., HILLS, N.K., SAVER, J.L. and JOHNSTON, S.C., 2006. Frequency and determinants of pneumonia and urinary tract infection during stroke hospitalization. *Journal of Stroke and Cerebrovascular Disease*, **15**(5), pp. 209-213.

PATTERSON, J.R. and GRABOIS, M., 1986. Locked-in syndrome: a review of 139 cases. *Stroke,* **17**(4), pp. 758-764.

PETERSEN, O.H., 2007. *Lecture notes: Human physiology.* 5 edn. Oxford: Blackwell Publishing.

PITTS, T., BOLSER, D., ROSENBEK, J., TROCHE, M., OKUN, M.S. and SAPIENZA, C., 2009. Impact of expiratory muscle strength training on voluntary cough and swallow function in Parkinson disease. *CHEST Journal,* **135**(5), pp. 1301-1308.

POLKEY, M.I., GREEN, M. and MOXHAM, J., 1995. Measurement of respiratory muscle strength. *Thorax,* **50**(11), pp. 1131-1135.

POLKEY, M.I. and MOXHAM, J., 2004. Improvement in volitional tests of muscle function alone may not be adequate evidence that inspiratory muscle training is effective. *European Respiratory Journal,* **23**(1), pp. 5-6.

POLLOCK, R.D., RAFFERTY, G.F., MOXHAM, J. and KALRA, L., 2013. Respiratory muscle strength and training in stroke and neurology: a systematic review. *International Journal of Stroke,* **8**(2), pp. 124-130.

PRASS, K., MEISEL, C., HÖFLICH, C., BRAUN, J., HALLE, E., WOLF, T., RUSCHER, K., VICTOROV, I.V., PRILLER, J., DIRNAGL, U., VOLK, H. and MEISEL, A., 2003. Stroke-induced immunodeficiency promotes spontaneous bacterial infections and is mediated by sympathetic activation reversal by poststroke T helper cell type 1–like immunostimulation. *The Journal of Experimental Medicine*, **198**(5), pp. 725-736.

PRZEDBORSKI, S., BRUNKO, E., HUBERT, M., MAVROUDAKIS, N. and DE BEYL, D.Z., 1988. The effect of acute hemiplegia on intercostal muscle activity. *Neurology,* **38**(12), pp. 1882-1882.

RAJ, A.A. and BIRRING, S.S., 2007. Clinical assessment of chronic cough severity. *Pulmonary Pharmacology and Therapeutics,* **20**(4), pp. 334-337.

RAMSEY, D.J.C., SMITHARD, D.G. and KALRA, L., 2003. Early assessments of dysphagia and aspiration risk in acute stroke patients. *Stroke*, **34**(5), pp. 1252-1257.

RANKIN, G. and STOKES, M., 1998. Reliability of assessment tools in rehabilitation: an illustration of appropriate statistical analyses. *Clinical Rehabilitation*, **12**(3), pp. 187-199.

REID, W.D. and DECHMAN, G., 1995. Considerations when testing and training the respiratory muscles. *Physical Therapy,* **75**(11), pp. 971-982.

REID, W.D. and SAMRAI, B., 1995. Respiratory muscle training for patients with chronic obstructive pulmonary disease. *Physical Therapy,* **75**(11), pp. 996-1005.

ROMER, L.M. and MCCONNELL, A.K., 2003. Specificity and reversibility of inspiratory muscle training. *Medicine and Science in Sports and Exercise,* **35**(2), pp. 237-244.

ROTH, E.J., LOVELL, L., HARVEY, R.L., HEINEMANN, A.W., SEMIK, P. and DIAZ, S., 2001. Incidence of and risk factors for medical complications during stroke rehabilitation. *Stroke,* **32**(2), pp. 523-529.

ROYAL COLLEGE OF PHYSICIANS, 2011. *National sentinel stroke clinical audit 2010 round 7.* London: Royal College of Physicians.

ROYAL COLLEGE OF PHYSICIANS, 2009. *National sentinel stroke audit phase II (clinical audit) 2008.* London: Royal College of Physicians.

SALA, R., MUNTO, M.J., DE LA CALLE, J., PRECIADO, I., MIRALLES-PEREZ, M.T., CORTES, A., MOLLA, R. and ALCAIDE, M., 1998. Changes in swallowing after cerebrovascular accidents: incidence, natural history and repercussions on nutritional status, morbidity and mortality. *Revue Neurologique,* **27**(159), pp. 759-766.

SANCHO, J., SERVERA, E., VERGARA, P. and MARIN, J., 2003. Mechanical Insufflation-Exsufflation vs. Tracheal Suctioning via Tracheostomy Tubes for Patients with Amyotrophic Lateral Sclerosis: A Pilot Study. *American Journal of Physical Medicine and Rehabilitation,* **82**(10), pp. 750-753.

SANCHO, J., SERVERA, E., DIAZ, J. and MARIN, J., 2007. Predictors of ineffective cough during a chest infection in patients with stable amyotrophic lateral sclerosis. *American Journal of Respiratory and Critical Care Medicine,* **175**(12), pp. 1266-1271.

SANCHO, J., SERVERA, E., DIAZ, J. and MARIN, J., 2004. Comparison of peak cough flows measured by pneumotachograph and a portable peak flow meter. *American Journal of Physical Medicine and Rehabilitation,* **83**(8), pp. 608-612.

SANTAMARIA, J. and RUIZ, C., 1988. Diaphragmatic elevation in stroke. *European Neurology,* **28**(2), pp. 81-83.

SAPOSNIK, G., HILL, M.D., O'DONNELL, M., FANG, J., HACHINSKI, V. and KAPRAL, M.K., 2008. Variables associated with 7-day, 30-day, and 1-year fatality after ischemic stroke. *Stroke,* **39**(8), pp. 2318-2324.

SELLARS, C., BOWIE, L., BAGG, J., SWEENEY, M.P., MILLER, H., TILSTON, J., LANGHORNE, P. and STOTT, D.J., 2007. Risk factors for chest infection in acute stroke: A prospective cohort study. *Stroke,* **38**(8), pp. 2284-2291.

SHAHEEN, H.A., KHORSHIED, M., EL-SAYED, M.A. and TAHA, M.A., 2012. Post stroke infection frequency and immunosuppression contribution. *Egyptian Journal of Neurology, Psychiatry and Neurosurgery,* **49**(3), pp. 239-244.

SHINOHARA, Y. and ORIGASA, H., 2012. Post-stroke pneumonia prevention by angiotensin-converting enzyme inhibitors: results of a meta-analysis of five studies in Asians. *Advances in Therapy,* **29**(10), pp. 900-912.

SICCOLI, M.M., VALKO, P.O., HERMANN, D.M. and BASSETTI, C.L., 2008. Central periodic breathing during sleep in 74 patients with acute ischemic stroke. Neurogenic and cardiogenic factors. *Journal of Neurology,* **255**(11), pp. 1687-1692.

SIECK, G.C. and GRANSEE, H.M., 2012. Respiratory muscles: Structure, function, and regulation. In: D.N. GRANGER and J. GRANGER, eds, *Colloquium series on integrated systems physiology: From molecule to function.* San Rafael, California: Morgan & Claypool Life Sciences, pp. 1-88.

SILVER, F.L., NORRIS, J.W., LEWIS, A.J. and HACHINSKI, V.C., 1984. Early mortality following stroke: a prospective review. *Stroke,* **15**(3), pp. 492-496.

SILVERMAN, E.P., CARNABY-MANN, G., PITTS, T., DAVENPORT, P., OKUN, M.S. and SAPIENZA, C., 2014. Concordance and discriminatory power of cough measurement devices for individuals with Parkinson's disease. *CHEST Journal*, **145**(5), pp. 1089-1096.

SIMILOWSKI, T., CATALA, M., RANCUREL, G. and DERENNE, J.P., 1996. Impairment of central motor conduction to the diaphragm in stroke. *American Journal of Respiratory and Critical Care Medicine,* **154**(2), pp. 436-441.

SINGH, P., MURTY, G.E., MAHAJAN, R.P., KNIGHTS, D. and AITKENHEAD, A.R., 1994. The tussometer: Accuracy and reproducibility. *British Journal of Anaesthesia,* **73**(2), pp. 145-148.

SINGH, S. and HAMDY, S., 2006. Dysphagia in stroke patients. *Postgraduate Medical Journal,* **82**(968), pp. 383-391.

SIVASOTHY, P., BROWN, L., SMITH, I.E. and SHNEERSON, J.M., 2001. Effect of manually assisted cough and mechanical insufflation on cough flow of normal subjects, patients with chronic obstructive pulmonary disease (COPD), and patients with respiratory muscle weakness. *Thorax,* **56**(6), pp. 438-444.

SMELTZER, S.C., LEVIETES, M.H. and COOK, S.D., 1996. Expiratory training in multiple sclerosis. *Archives of Physical Medicine and Rehabilitation,* **77**(9), pp. 909-912.

SMITH HAMMOND, C.A., GOLDSTEIN, L.B., ZAJAC, D.J., GRAY, L., DAVENPORT, P.W. and BOLSER, D.C., 2001. Assessment of aspiration risk in stroke patients with quantification of voluntary cough. *Neurology,* **56**(4), pp. 502-506.

SMITH HAMMOND, C.A., GOLDSTEIN, L.B., HORNER, R.D., YING, J., GRAY, L., GONZALEZ-ROTHI, L. and BOLSER, D.C., 2009. Predicting aspiration in patients with ischemic stroke: Comparison of clinical signs and aerodynamic measures of voluntary cough. *CHEST Journal,* **135**(3), pp. 769-777.

SMITH, M., 1962. The effect of hemiplegia on the diaphragm. *American Review of Respiratory Disease,* **89**, pp. 450-452.

SMITHARD, D.G., O'NEILL, P.A., PARK, C., MORRIS, J., WYATT, R., ENGLAND, R. and MARTIN, D.F., 1996. Complications and outcome after acute stroke: Does dysphagia matter? *Stroke,* **27**(7), pp. 1200-1204.

SMITHARD, D.G., SMEETON, N.C. and WOLFE, C.D.A., 2007. Long-term outcome after stroke: does dysphagia matter? *Age and Ageing,* **36**(1), pp. 90-94.

STALEY, K., 2009. *Exploring impact: public involvement in NHS, public health and social care research.* Eastleigh: INVOLVE.

STATACORP, 2013, Stata Base Reference Manual. Release 14. [Homepage of Stata Press], [Online]. Available at: http://www.stata.com/manuals13/ricc.pdf#riccRemarksandexamples [Accessed on: 23 July 2014].

STATACORP, 2011, Stata Multiple-Imputation Reference Manual [Homepage of Stata Press], [Online]. Available at: http://www.stata-press.com/manuals/stata12/multiple-imputation-reference-manual/ [Accessed on: 09 May 2014].

STEPHENS, R.E., ADDINGTON, W.R. and WIDDICOMBE, J.G., 2003. Effect of acute unilateral middle cerebral artery infarcts on voluntary cough and the laryngeal cough reflex. *American Journal of Physical Medicine and Rehabilitation,* **82**(5), pp. 379-383.

STREINER, D.L. and NORMAN, G.R., 2008. *Health measurement scales. A practical guide to their development and use.* 4th edn. Oxford: Oxford University Press.

SUAREZ, A.A., PESSOLANO, F.A., MONTEIRO, S.G.P.T., FERREYRA, G.P.T., CAPRIA, M.E.P.T., MESA, L. and DUBROVSKY, A.E.L., 2002. Peak flow and peak cough flow in the evaluation of expiratory muscle weakness and bulbar impairment in patients with neuromuscular disease. *American Journal of Physical Medicine and Rehabilitation,* **81**(7), pp. 506-511.

SUNGER, K., POWLEY, W., KELSALL, A., SUMNER, H., MURDOCH, R. and SMITH, J.A., 2013. Objective measurement of cough in otherwise healthy volunteers with acute cough. *European Respiratory Journal,* **41**(2), pp. 277-284.

SUTBEYAZ, S.T., KOSEOGLU, F., INAN, L. and COSKUN, O., 2010. Respiratory muscle training improves cardiopulmonary function and exercise tolerance in subjects with subacute stroke: A randomized controlled trial. *Clinical Rehabilitation,* **24**(3), pp. 240-250.

SYABBALO, N., 1998. Assessment of respiratory muscle function and strength. *Postgraduate Medical Journal,* **74**(870), pp. 208-215.

TAYLOR, J.R., 1997. *An Introduction to error analysis.* 2nd edn. Sausalito, CA: University Science Books.

TAYLOR, P.N., TROMANS, A.M., HARRIS, K.R. and SWAIN, I.D., 2002. Electrical stimulation of abdominal muscles for control of blood pressure and augmentation of cough in a C3/4 level tetraplegic. *Spinal Cord,* **40**(1), pp. 34-36.

TEIXEIRA-SALMELA, L., PARREIRA, V.F., BRITTO, R.R., BRANT, T.C., INACIO, A.P., ALCANTARA, T.O. and CARVALHO, I.F., 2005. Respiratory pressures and thoracoabdominal motion in community-dwelling chronic stroke survivors. *Archives of Physical Medicine and Rehabilitation,* **86**(10), pp. 1974-1978.

TEIXEIRA-SALMELA, L.F., BRITTO, R.R., PARREIRA, V.F., TORRES, J.L., REZENDE, N.R. and MARINHO, K.C., 2010. Effects of inspiratory muscular training in chronic stroke survivors, *45th Annual Congress of the Canadian Neurological Sciences Federation*, 05/01 2010, pp. S27-S28.

TERAMOTO, S., 2009. Novel preventive and therapeutic strategy for post-stroke pneumonia. *Expert Review of Neurotherapeutics,* **9**(8), pp. 1187-1200.

TONG, X., KUKLINA, E.V., GILLESPIE, C. and GEORGE, M.G., 2010. Medical complications among hospitalizations for ischemic stroke in the United States from 1998 to 2007. *Stroke,* **41**(5), pp. 980-986.

TOWNSEND, N., WICKRAMASINGHE, K., BHATNAGAR, P., SMOLINA, K., NICHOLS, M., LUENGO-FERNANDEZ, R. and RAYNER, M., 2012. *Coronary heart disease statistics 2012 edition.* London: British Heart Foundation.

TREBBIA, G., LACOMBE, M., FERMANIAN, C., FALAIZE, L., LEJAILLE, M., LOUIS, A., DEVAUX, C., RAPHAEL, J.C. and LOFASO, F., 2005. Cough determinants in patients with neuromuscular disease. *Respiratory Physiology and Neurobiology,* **146**(2–3), pp. 291-300.

UPADYA, A., THOREVSKA, N., SENA, K.N., MANTHOUS, C. and AMOATENG-ADJEPONG, Y., 2004. Predictors and consequences of pneumonia in critically ill patients with stroke. *Journal of Critical Care,* **19**(1), pp. 16-22.

URBAN, P.P., MORGENSTERN, M., BRAUSE, K., WICHT, S., VUKUREVIC, G., KESSLER, S. and STOETER, P., 2002. Distribution and course of cortico-respiratory projections for voluntary activation in man. *Journal of Neurology,* **249**(6), pp. 735-744.

VERGES, S., BOUTELLIER, U. and SPENGLER, C.M., 2008. Effect of respiratory muscle endurance training on respiratory sensations, respiratory control and exercise performance: A 15-year experience. *Respiratory Physiology and Neurobiology,* **161**(1), pp. 16-22.

VERHAGEN, A.P., DE VET, H.C.W., DE BIE, R.A., KESSELS, A.G.H., BOERS, M., BOUTER, L.M. and KNIPSCHILD, P.G., 1998. The Delphi list: A criteria list for quality assessment of randomized clinical trials for conducting systematic reviews developed by Delphi consensus. *Journal of Clinical Epidemiology*, **51**(12), pp. 1235-1241.

VERNINO, S., BROWN, R.D., SEJVAR, J.J., SICKS, J.D., PETTY, G.W. and O'FALLON, W.M., 2003. Cause-specific mortality after first cerebral infarction: A population-based study. *Stroke,* **34**(8), pp. 1828-1832.

VIANELLO, A., CORRADO, A., ARCARO, G., GALLAN, F., ORI, C., MINUZZO, M. and BEVILACQUA, M., 2005. Mechanical insufflation-exsufflation improves outcomes for neuromuscular disease patients with respiratory tract infections. *American Journal of Physical Medicine and Rehabilitation,* **84**(2), pp. 83-88.

VIITANEN, M., WINBLAD, B. and ASPLUND, K., 1987. Autopsy-verified causes of death after stroke. *Acta Medica Scandinavica,* **222**, pp. 401-408.

VOYVODA, N., YUCEL, C., KARATAS, G., OGUZULGEN, I and OKTAR, S., 2012. An evaluation of diaphragmatic movements in hemiplegic patients. *The British Journal of Radiology,* **85**(1012), pp. 411-414.

WALTER, U., KNOBLICH, R., STEINHAGEN, V., DONAT, M., BENECKE, R. and KLOTH, A., 2007. Predictors of pneumonia in acute stroke patients admitted to a neurological intensive care unit. *Journal of Neurology,* **254**(10), pp. 1323-1329.

WARD, C.H., 2012. *Respiratory impairment in stroke patients: Lung function, respiratory muscles, voluntary and reflex cough*, Kings College London, School of Medicine.

WARD, K., SEYMOUR, J., STEIER, J., JOLLEY, C.J., POLKEY, M.I., KALRA, L. and MOXHAM, J., 2010. Acute ischaemic hemispheric stroke is associated with impairment of reflex in addition to voluntary cough. *European Respiratory Journal,* **36**(6), pp. 1383-1390.

WEINER, P. and MCCONNELL, A., 2005. Respiratory muscle training in chronic obstructive pulmonary disease: inspiratory, expiratory, or both? *Current Opinion in Pulmonary Medicine,* **11**(2), pp. 140-144.

WESTENDORP WILLEKE, F., VERMEIJ, J., VERMEIJ, F., DEN HERTOG HELEEN, M., DIPPEL DIEDERIK, W.J., VAN DE BEEK, D. and NEDERKOORN PAUL, J., 2012. Antibiotic therapy for preventing infections in patients with acute stroke. *Cochrane Database of Systematic Reviews,* (1), Art.No.:CD008530.

WHITE, I.R., ROYSTON, P. and WOOD, A.M., 2011. Multiple imputation using chained equations: Issues and guidance for practice. *Statistics in Medicine,* **30**(4), pp. 377-399.

WIDDICOMBE, J. and FONTANA, G., 2006. Cough: What's in a name? *European Respiratory Journal,* **28**(1), pp. 10-15.

WIDDICOMBE, J.G., 1998. Afferent receptors in the airways and cough. *Respiration Physiology,* **114**(1), pp. 5-15.

WIDDICOMBE, J.G., ADDINGTON, W.R., FONTANA, G.A. and STEPHENS, R.E., 2011. Voluntary and reflex cough and the expiration reflex: Implications for aspiration after stroke. *Pulmonary Pharmacology and Therapeutics,* **24**(3), pp. 312-317.

WIDDICOMBE, J., 2001. Airway receptors. *Respiration Physiology,* **125**(1–2), pp. 3-15.

WIDDICOMBE, J. and SINGH, V., 2006. Physiological and pathophysiological down-regulation of cough. *Respiratory Physiology and Neurobiology,* **150**(2–3), pp. 105-117.

WILSON, R.D., 2012. Mortality and cost of pneumonia after stroke for different risk groups. *Journal of Stroke and Cerebrovascular Disease,* **21**(1), pp. 61-67.

WORLD HEALTH ORGANIZATION, 1971. *Cerebrovascular diseases: prevention, treatment and rehabilitation, report of a WHO meeting.* Geneva: World Health Organization.

WRIGHT, M.B. and MCKERROW, C.B., 1959. Maximum forced expiratory flow rate as a measure of ventilatory capacity. *British Medical Journal,* **2**, pp. 1041-1047.

XIAO, Y., LUO, M., WAND, H. and LUO, H., 2012. Inspiratory muscle training for the recovery of function after stroke. *Cochrane Database of Systematic Reviews*, (5), Art.No.:CD009360.

YEH, S., HUANG, K., WANG, T., CHEN, Y., CHEN, C., TANG, S., TSAI, L., YIP, P. and JENG, J., 2011. Dysphagia screening decreases pneumonia in acute stroke patients admitted to the stroke intensive care unit. *Journal of the Neurological Sciences,* **306**(1), pp. 38-41.

YOON, J., PARK, J., LEE, D. and ROH, H., 2011. Comparisons of respiratory function and activities of daily living between spinal cord injury and stroke patients and normal elderly people. *Journal of Physical Therapy Science,* **24**, pp. 465-469.

YOUSAF, N., MONTEIRO, W., MATOS, S., BIRRING, S.S. and PAVORD, I.D., 2013. Cough frequency in health and disease. *European Respiratory Journal,* **41**(1), pp. 241-243.

ZHOU, Z., VINCENT, F., SALLE, J., ANTONINI, M., ALIAMUS, V. and DAVIET, J., 2012. Acute stroke phase voluntary cough and correlation with maximum phonation time. *American Journal of Physical Medicine and Rehabilitation,* **91**(6), pp. 494-500.

Appendices

Appendix 1

Studies reporting the incidence of pneumonia in stroke survivors

Author, year and country	Study design	Setting	Total sample (n)	Criteria for pneumonia diagnosis	Observation period for incidence of pneumonia	Incidence of pneumonia		Comments
						n	%	
Yeh *et al.* 2011 Taiwan	Prospective Single-centre	Intensive care	176	a	Hospital admission	100	56.8%	-
Walter *et al.* 2007 Germany	Prospective Single-centre	Intensive care	236	a	Intensive care admission	51	21.6%	Ischemic stroke only
Upadya *et al.* 2004 United States	Retrospective Single-centre	Intensive care	55	a	Hospital admission	26	47.3%	-
Hilker *et al.*	Prospective	Intensive care	124	a	Intensive care admission	26	21.0%	-

2003 Germany	Single-centre							
Kwon *et al.* 2006 South Korea	Prospective Single-centre	Intensive care and acute	268	a	30 days	47	16.4%	Included 31 mechanically ventilated patients, of whom 24 had pneumonia
Hoffmann *et al.* 2012 Germany	Retrospective Multi-centre	Acute	60,420	a	Hospital admission	-	7.6%	Ischemic stroke only
Shaheen *et al.* 2012 Egypt	Prospective Single-centre	Acute	25	a	2 weeks from stroke	7	24.0%	Ischemic stroke only
Wilson 2012 United States	Retrospective Multi-centre	Acute	18,3976	c	Hospital admission	-	8.1%	-
Finlayson *et al.* 2011 Canada	Retrospective Multi-centre	Acute	8,251	a	30 days from stroke	587	7.1%	Ischemic stroke only
Ingeman *et al.* 2011	Retrospective Multi-centre	Acute	11,757	a	Hospital admission	-	8.8%	-

Denmark								
Koennecke *et al.* 2011 Germany	Prospective Multi-centre	Acute	16,518	a	Hospital admission	1,269	7.7%	-
Royal College of Physicians 2011 United Kingdom	Retrospective Multi-centre	Acute	11,353	d	Hospital admission	-	13%	Audit data from England, Wales and Northern Ireland
Chumbler *et al.* 2010 United States	Retrospective Multi-centre	Acute	1,363	b	Hospital admission	142	10.4%	-
Lakshminarayan *et al.* 2010 United States	Retrospective Multi-centre	Acute	18,017	b	Hospital admission	711	3.9%	Excluding patients with contraindications for dysphagia screening and patients remaining nil by mouth
Tong *et al.* 2010 United States	Retrospective, Multi-centre	Acute	1,150,336	c	Hospital admission	-	3.0%	Ischemic stroke only
Royal College of Physicians	Retrospective Multi-centre	Acute	11,369	d	Hospital admission	-	16%	Audit data from England, Wales and Northern Ireland

2009 United Kingdom								
Hong *et al.* 2008 South Korea	Prospective Multi-centre	Acute	1,254	a	Hospital admission	151	12.0%	Ischemic stroke only
Indredavik *et al.* 2008 Norway	Prospective Single-centre	Acute	489	a	7 days from stroke	55	11.2%	Subgroup of 244 patients observed for 3 months from stroke, with pneumonia incidence of 17.2%
Masiero *et al.* 2008 Italy	Prospective Single centre	Acute	67	a	6 months from stroke	9	13.4%	Stroke patients with new oropharyngeal dysphagia
Qureshi *et al.* 2007 United States	Retrospective Multi-centre	Acute	1,736,352 in 1990/1991 1,958,018 in 2000/2001	c	Hospital admission	-	2.1% in 1990/1991 2.2% in 2000/2001	-
Sellars *et al.* 2007 United Kingdom	Prospective Single-centre	Acute	412	a, b	3 months from stroke	78 with pneumonia 82 with suspected pneumonia	18.9% with pneumonia 19.9% with suspected pneumonia	Distinguished between confirmed pneumonia (a) and suspected pneumonia (b)

Ovbiagele *et al.* 2006 United States	Prospective Multi-centre	Acute	660	d	Hospital admission	66	10.0%	Ischemic stroke only
Bae *et al.* 2005 South Korea	Prospective, Single-centre	Acute	579	a	Hospital admission	-	10.7%	Ischemic stroke only
Hinchey *et al.* 2005 United States	Pro- and retrospective Multi-centre	Acute	2532	a	Hospital admission	-	4.5%	Ischemic stroke only
Aslanyan *et al.* 2004 Europe (Finland, Germany, United Kingdom)	Retrospective Multi-centre	Acute	1,455	d	3 months from stroke	198	13.6%	Previously independent acute stroke patients; data from a randomised controlled trial
Dziewas *et al.* 2004 Germany	Prospective Single-centre	Acute	100	a	Hospital admission	44	44.0%	Acute stroke patients with naso-gastric tube due to failed swallowing screen
Heuschmann *et al.* 2004	Prospective Multi-centre	Acute	13,440	a	Hospital admission	-	6.0%	Ischemic stroke only

Germany								
Katzan *et al.* 2003 United States	Retrospective Multi-centre	Acute	14,293	c	Hospital admission	986	6.9%	-
Weimar *et al.* 2002 Germany	Prospective Multi-centre	Acute	3,866	a	7 days from stroke	-	7.4%	Ischemic stroke only
Grau *et al.* 1999 Germany	Prospective Single centre	Acute	119	a	Hospital admission	12	10.1%	-
Mann *et al.* 1999 Australia	Prospective Single-centre	Acute	128	a	6 months from stroke	26	20.0%	Excluding unconscious patients and patients with previous swallowing problems
Tirschwell & Kukull 1999 United States	Retrospective Multi-centre	Acute	4,757	c	Hospital admission	-	7.1%	Ischemic stroke only
Davenport *et al.* 1996	Prospective Single-centre	Acute	607	a	Hospital admission	70	12.0%	-

United Kingdom								
Addington *et al.* 2005 United States	Prospective Multi-centre	Acute and rehabilitation	818	a	Acute and rehabilitation admission	35	4.3%	-
Langhorne *et al.* 2000 United Kingdom	Prospective Multi-centre	Acute and rehabilitation	311	a	30 months from stroke	-	22% from hospital admission to 2 months 13% from discharge to 6 months 23% from 6 months to 18 months 29% from 18 months to 30 months	-
Marciniak *et al.* 2009 United States	Retrospective Single-centre	Rehabilitation	1,099	b	Rehabilitation admission	36	3.3%	-
Roth *et al.* 2001 United States	Prospective Single-centre	Rehabilitation	1,029	d	Rehabilitation admission	42	4.0%	-

Teasell *et al.* 1996 Canada	Retrospective Single-centre	Rehabilitation	441	a	Acute and rehabilitation admission	12	2.7%	Patients admitted for rehabilitation within 4 months of stroke
Kalra *et al.* 1995 United Kingdom	Retrospective Single-centre	Rehabilitation	245	a	Rehabilitation admission	10 of 124 patients receiving rehabilitation on a stroke unit 19 of 121 patients receiving rehabilitation on general hospital wards	8% of patients receiving rehabilitation on a stroke unit 16% of patients receiving rehabilitation on general hospital wards	Data from a randomised trial
Dromerick & Reding 1994 United States	Retrospective Single-centre	Rehabilitation	100	b	Rehabilitation admission	7	7.0%	-
Holas *et al.* 1994 United States	Prospective Single-centre	Rehabilitation	114	a	Rehabilitation admission	9	7.9%	Stroke patients with dysphagia confirmed on videofluoroscopy
Dobkin	Retrospective	Rehabilitation	200	d	Rehabilitation admission	4	2.0%	-

1987	Single centre	
United States		

a, diagnosis of pneumonia made based on clinical criteria, e.g. according to Mann (Mann *et al.* 1999) or the US Centres for Disease Control (Garner *et al*. 1988)

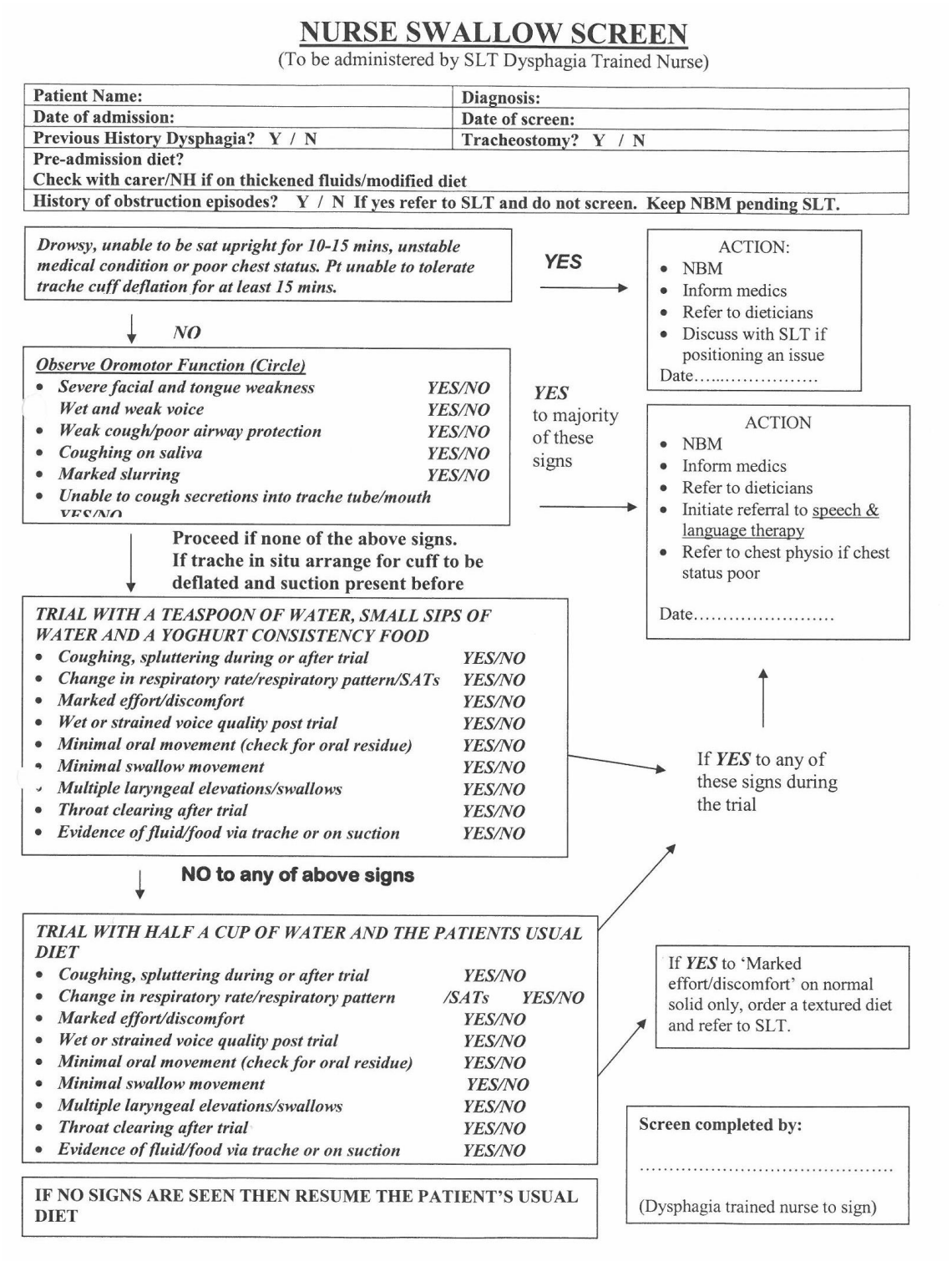
b, diagnosis of pneumonia as per documented clinical diagnosis

c, diagnosis of pneumonia as per diagnostic codes (International Classification of Diseases, ICD)

d, criteria for diagnosis of pneumonia not given

Appendix 2

Clinical algorithm for routine swallow screen



Appendix 3

Pneumonia definition

The diagnostic criteria for pneumonia and operational procedures for detecting pneumonia in the present studies are given below. The diagnostic criteria, although aligned with previous criteria applied in clinical research, can be regarded as 'ad hoc' criteria (Kishore *et al.* 2015). For data collection purposes, presence of pneumonia was determined either from the treating physician's diagnosis or from participants' self-report. The methodological limitations to this approach are acknowledged, although there is currently no recommended standard method for diagnosing pneumonia in clinical stroke research (Kishore *et al*. 2015).

Diagnostic criteria	Operational procedure
Raised temperature (>37.5 °C on two consecutive measurements or a single measurement of >38 °C) and Chest symptoms (dyspnoea, tachypnoea, productive cough, reduced oxygen saturation, inspiratory crackles, bronchial breathing) and ≥1 of the following: • White cell count >11,000/mL • Pulmonary infiltrate on chest radiograph • Positive microbiology cultures	Yes to any one of the following: • Documented medical diagnosis of pneumonia / chest infection • Prescription of antibiotics for pneumonia / chest infection • Hospital admission for pneumonia / chest infection • Self-reported pneumonia / chest infection

Appendix 4

Stata outputs for diagnostic statistics (chapter 6)

1) Model for interaction between swallow safety and voluntary cough PECF

```
. xi: logistic RegPneu4Weeks i.Swallowsafety1safe2unsafe*VCPEFRbaseline
i.Swallowsafe~e   _ISwallowsa_1-2     (naturally coded; _ISwallowsa_1 omitted)
i.Swal~e*VCPE~e   _ISwaXVCPEF_#       (coded as above)

Logistic regression                               Number of obs   =         72
                                                  LR chi2(3)      =      17.46
                                                  Prob > chi2     =     0.0006
Log likelihood = -25.273272                       Pseudo R2       =     0.2567
```

RegPneu4Weeks	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
_ISwallowsa_2	83.37231	161.9995	2.28	0.023	1.849546	3758.189
VCPEFRbaseline	1.00017	.0027795	0.06	0.951	.9947371	1.005632
_ISwaXVCPEF_2	.9934487	.0040945	-1.59	0.111	.9854559	1.001506
_cons	.0493108	.0828928	-1.79	0.073	.0018283	1.329952

```
. estat gof

Logistic model for RegPneu4Weeks, goodness-of-fit test

       number of observations =        72
 number of covariate patterns =        72
             Pearson chi2(68) =        68.55
                  Prob > chi2 =         0.4585

. estat gof, group(10)

Logistic model for RegPneu4Weeks, goodness-of-fit test

  (Table collapsed on quantiles of estimated probabilities)

       number of observations =        72
             number of groups =        10
     Hosmer-Lemeshow chi2(8) =         5.11
                  Prob > chi2 =         0.7454
```

```
. lstat

Logistic model for RegPneu4Weeks
```

Classified	True D	True ~D	Total
+	6	4	10
-	7	55	62
Total	13	59	72

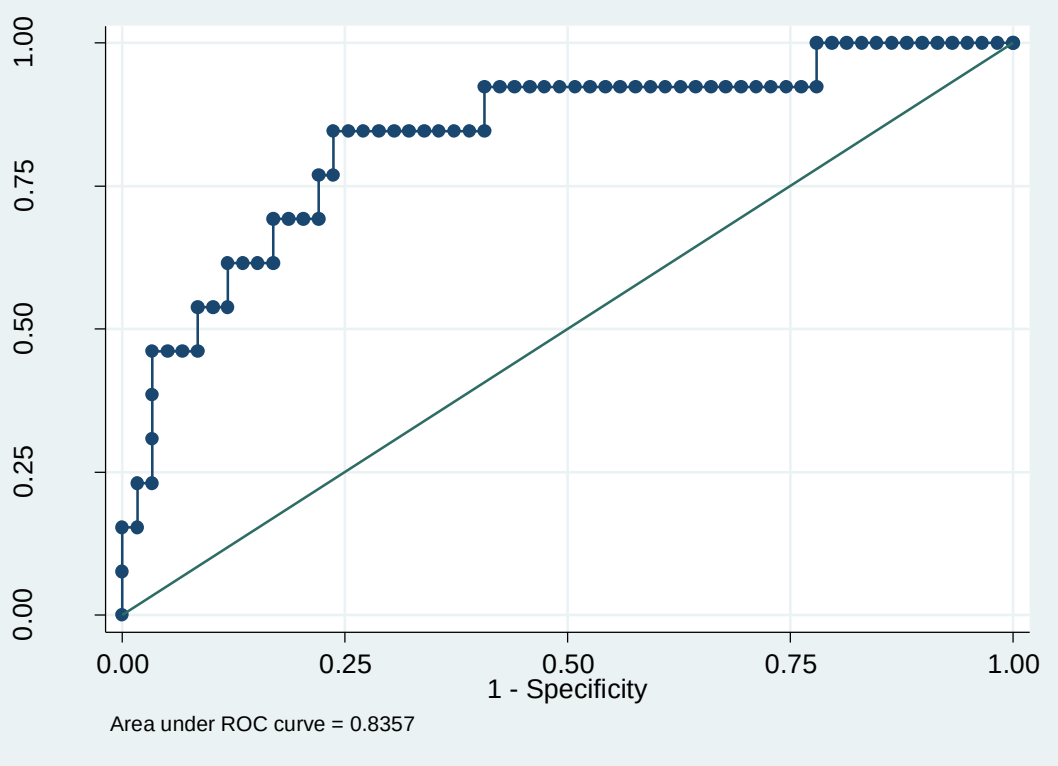
```
Classified + if predicted Pr(D) >= .5
True D defined as RegPneu4Weeks != 0
```

Sensitivity	Pr(+| D)	46.15%
Specificity	Pr(-|~D)	93.22%
Positive predictive value	Pr(D| +)	60.00%
Negative predictive value	Pr(~D| -)	88.71%
False + rate for true ~D	Pr(+|~D)	6.78%
False - rate for true D	Pr(-| D)	53.85%
False + rate for classified +	Pr(~D| +)	40.00%
False - rate for classified -	Pr(D| -)	11.29%
Correctly classified		84.72%

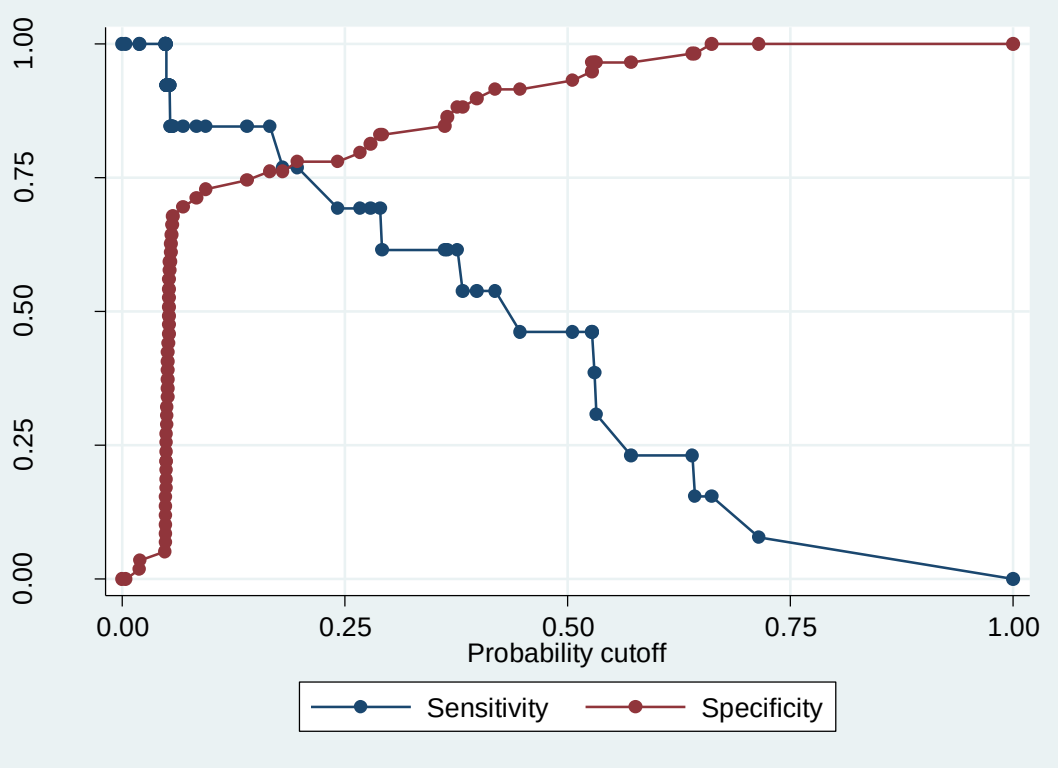
```
. lroc

Logistic model for RegPneu4Weeks

number of observations =        72
area under ROC curve   =    0.8357
```



```
. lsens
```



2) Model for interaction between swallow safety and reflex cough PECF

```
. xi: logistic RegPneu4Weeks i.Swallowsafety1safe2unsafe*RCPEFRbaseline
i.Swallowsafe~e   _ISwallowsa_1-2     (naturally coded; _ISwallowsa_1 omitted)
i.Swal~e*RCPE~e   _ISwaXRCPEF_#       (coded as above)

Logistic regression                               Number of obs   =         69
                                                  LR chi2(3)      =      11.28
                                                  Prob > chi2     =     0.0103
Log likelihood = -27.747566                       Pseudo R2       =     0.1690
```

RegPneu4Weeks	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
_ISwallowsa_2	42.85813	105.7364	1.52	0.128	.3404177	5395.781
RCPEFRbaseline	1.001937	.0066848	0.29	0.772	.9889199	1.015125
_ISwaXRCPEF_2	.9943651	.0075345	-0.75	0.456	.9797069	1.009243
_cons	.0311995	.0709666	-1.52	0.127	.0003614	2.693388

```
. estat gof
```

Logistic model for RegPneu4Weeks, goodness-of-fit test

```
       number of observations =        69
 number of covariate patterns =        69
             Pearson chi2(65) =        68.96
                  Prob > chi2 =         0.3452
```

```
. estat gof, group(10)
```

Logistic model for RegPneu4Weeks, goodness-of-fit test

```
  (Table collapsed on quantiles of estimated probabilities)

       number of observations =        69
             number of groups =        10
     Hosmer-Lemeshow chi2(8) =         4.00
                  Prob > chi2 =         0.8574
```

```
. lstat

Logistic model for RegPneu4Weeks
```

	True		
Classified	D	~D	Total
+	0	0	0
-	12	57	69
Total	12	57	69

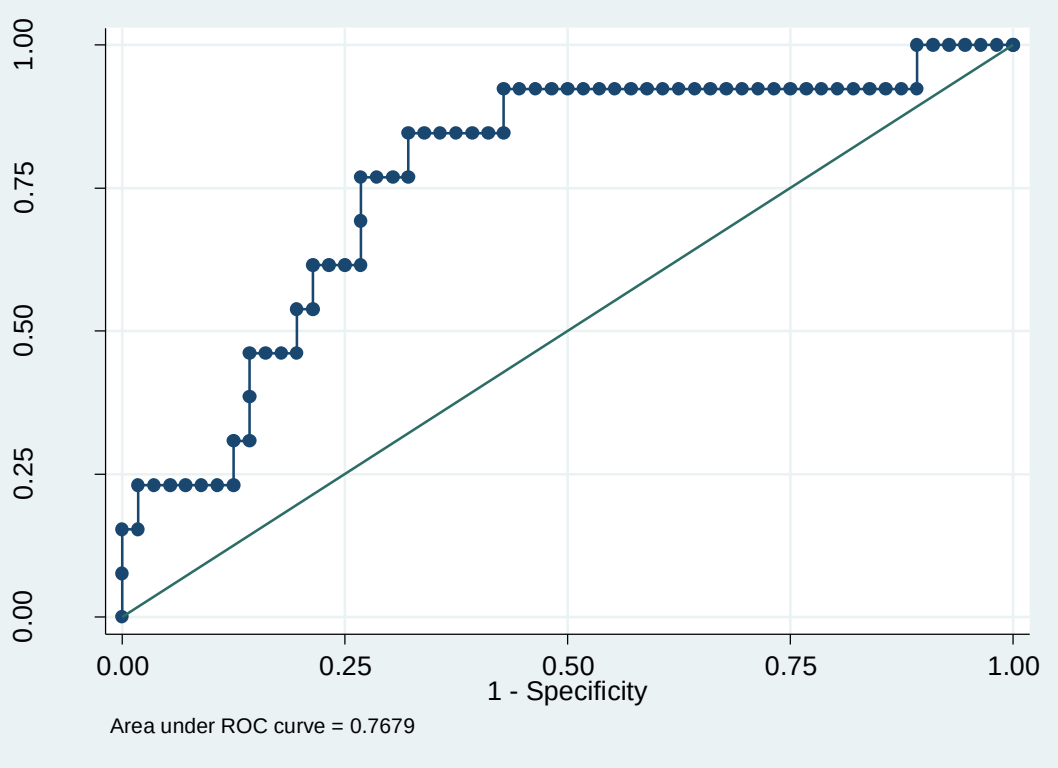
```
Classified + if predicted Pr(D) >= .5
True D defined as RegPneu4Weeks != 0
```

Sensitivity	Pr(+| D)	0.00%
Specificity	Pr(-|~D)	100.00%
Positive predictive value	Pr(D| +)	.%
Negative predictive value	Pr(~D| -)	82.61%
False + rate for true ~D	Pr(+|~D)	0.00%
False - rate for true D	Pr(-| D)	100.00%
False + rate for classified +	Pr(~D| +)	.%
False - rate for classified -	Pr(D| -)	17.39%
Correctly classified		82.61%

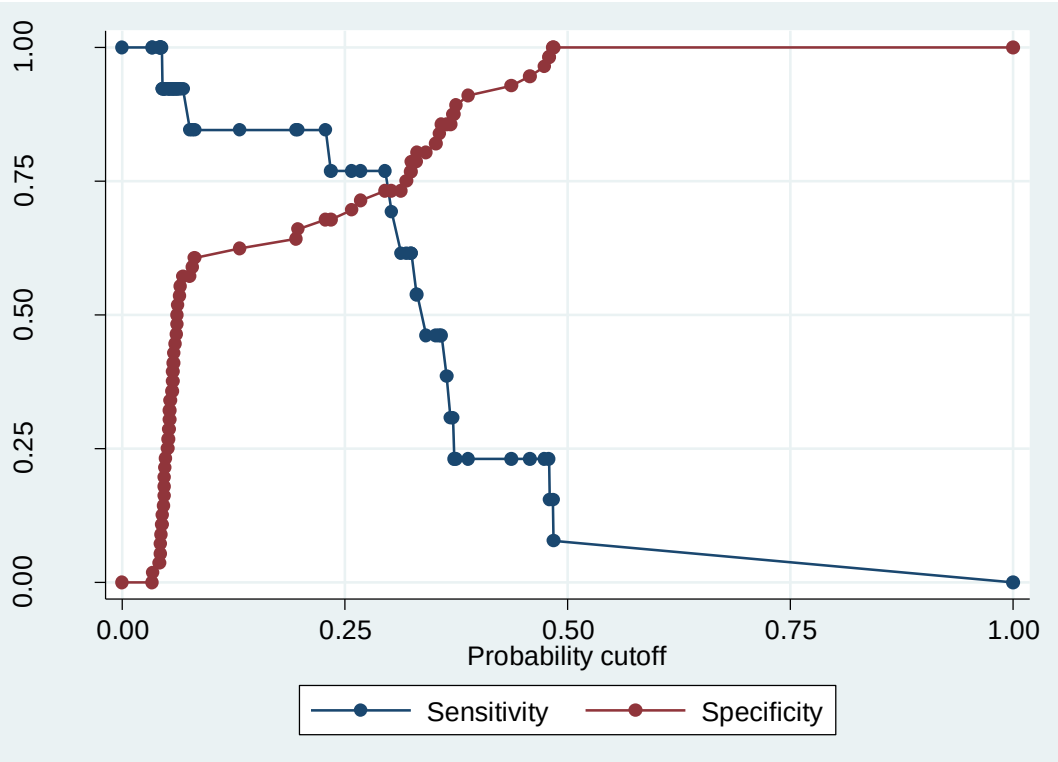
```
. lroc

Logistic model for RegPneu4Weeks

number of observations =        69
area under ROC curve   =    0.7679
```




```
. lsens
```



3) Model for interaction between swallow safety and voluntary cough PECF, adjusted for age, sex and stroke severity (admission NIHSS score)

```
. xi: logistic RegPneu4Weeks i.Swallowsafety1safe2unsafe*VCPEFRbaseline Age i.Sex NIHStrokeScore
i.Swallowsafe~e   _ISwallowsa_1-2     (naturally coded; _ISwallowsa_1 omitted)
i.Swal~e*VCPE~e   _ISwaXVCPEF_#       (coded as above)
i.Sex             _ISex_1-2           (naturally coded; _ISex_1 omitted)

Logistic regression                               Number of obs   =         72
                                                  LR chi2(6)      =      19.19
                                                  Prob > chi2     =     0.0039
Log likelihood = -24.406782                       Pseudo R2       =     0.2822
```

RegPneu4Weeks	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
_ISwallowsa_2	56.2039	105.2511	2.15	0.031	1.431367	2206.896
VCPEFRbaseline	1.001362	.0027475	0.50	0.620	.9959912	1.006761
_ISwaXVCPEF_2	.9938132	.0039866	-1.55	0.122	.9860304	1.001658
Age	1.036968	.031596	1.19	0.234	.9768539	1.100782
_ISex_2	1.289419	.9987285	0.33	0.743	.2825462	5.884347
NIHStrokeScore	1.041476	.0818154	0.52	0.605	.8928558	1.214835
_cons	.0017554	.0054221	-2.05	0.040	4.12e-06	.7475254

```
. estat gof

Logistic model for RegPneu4Weeks, goodness-of-fit test

       number of observations =        72
 number of covariate patterns =        72
             Pearson chi2(65) =        64.25
                  Prob > chi2 =         0.5029

. estat gof, group(10)

Logistic model for RegPneu4Weeks, goodness-of-fit test

  (Table collapsed on quantiles of estimated probabilities)

       number of observations =        72
             number of groups =        10
      Hosmer-Lemeshow chi2(8) =         4.66
                  Prob > chi2 =         0.7928
```

```
. lstat

Logistic model for RegPneu4Weeks
```

Classified	True D	True ~D	Total
+	7	4	11
-	6	55	61
Total	13	59	72

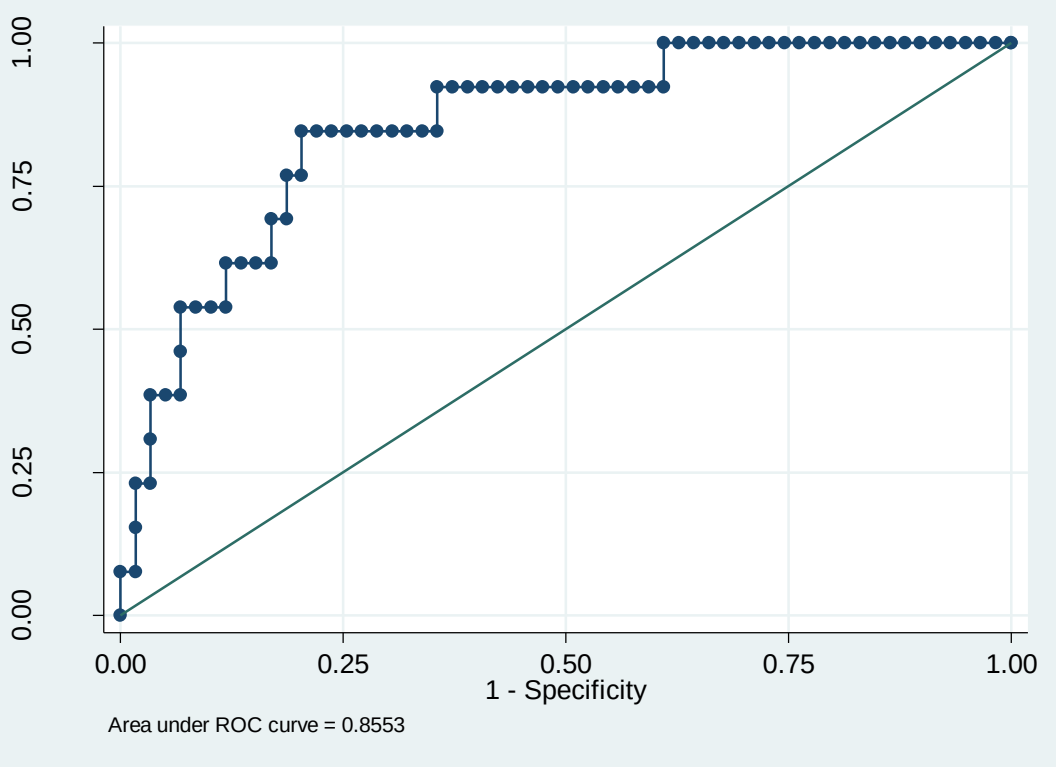
```
Classified + if predicted Pr(D) >= .5
True D defined as RegPneu4Weeks != 0
```

Sensitivity	Pr(+| D)	53.85%
Specificity	Pr(-|~D)	93.22%
Positive predictive value	Pr(D| +)	63.64%
Negative predictive value	Pr(~D| -)	90.16%
False + rate for true ~D	Pr(+|~D)	6.78%
False - rate for true D	Pr(-| D)	46.15%
False + rate for classified +	Pr(~D| +)	36.36%
False - rate for classified -	Pr(D| -)	9.84%
Correctly classified		86.11%

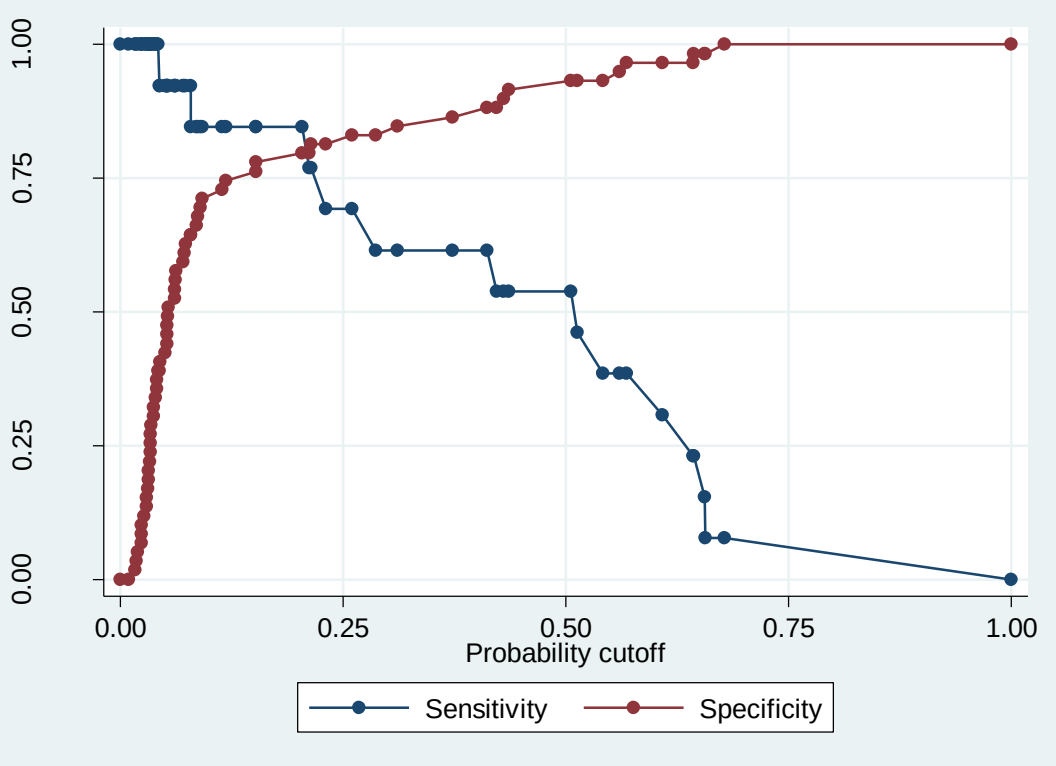
```
. lroc

Logistic model for RegPneu4Weeks

number of observations =       72
area under ROC curve   =   0.8553
```



```
. lsens
```


1.00
0.75
0.50
0.25
0.00
0.00
0.25
0.50
0.75
1.00
Probability cutoff
Sensitivity
Specificity

Appendix 5

Stata outputs for exact logistic regression model (chapter 6)

1) Pneumonia risk according to voluntary cough PECF in patients with low aspiration risk

```
. xi: exlogistic RegPneu4Weeks Cat24_VCPEFR if Swallowsafety==1, nolog

Exact logistic regression                       Number of obs =         39
                                                Model score   =   .0107891
                                                Pr >= score   =     0.9487
```

RegPneu4We~s	Odds Ratio	Suff.	2*Pr(Suff.)	[95% Conf.	Interval]
Cat24_VCPEFR	1.014311	23	0.8907	.7441706	1.326914

2) Pneumonia risk according to voluntary cough PECF in patients with high aspiration risk

```
. xi: exlogistic RegPneu4Weeks Cat24_VCPEFR if Swallowsafety==2, nolog

Exact logistic regression                       Number of obs =         33
                                                Model score   =   5.295465
                                                Pr >= score   =     0.0186
```

RegPneu4We~s	Odds Ratio	Suff.	2*Pr(Suff.)	[95% Conf.	Interval]
Cat24_VCPEFR	.7290848	62	0.0122	.5097866	.9487404

3) Pneumonia risk according to reflex cough PECF in patients with low aspiration risk

```
. xi: exlogistic RegPneu4Weeks Cat24_RCPEFR if Swallowsafety==1, nolog

Exact logistic regression                       Number of obs =         37
                                                Model score   =   .1004997
                                                Pr >= score   =     0.8589
```

RegPneu4We~s	Odds Ratio	Suff.	2*Pr(Suff.)	[95% Conf.	Interval]
Cat24_RCPEFR	1.10734	14	0.8378	.5522977	2.234083

4) Pneumonia risk according to reflex cough PECF in patients with high aspiration risk

```
. xi: exlogistic RegPneu4Weeks Cat24_RCPEFR if Swallowsafety==2, nolog

Exact logistic regression                       Number of obs =         32
                                                Model score   =   .7346174
                                                Pr >= score   =     0.4083
```

RegPneu4We~s	Odds Ratio	Suff.	2*Pr(Suff.)	[95% Conf.	Interval]
Cat24_RCPEFR	.869611	57	0.4481	.6047247	1.202335

Appendix 6

Letters from the Research Ethics Committees

NHS
National Research Ethics Service
NRES Committee London - Wandsworth

Room 4W012 4 Floor West
Charing Cross Hospital
Fulham Palace Road
London W6 8RF

Tel: 0203 311 7254

16 July 2011 (Reissued 15 August 2011)

Prof Lalit Kalra
Professor of Stroke Medicine
King's College London
Dept of Stroke Medicine
Academic Neurosciences Centre
King's College London
SE5 8AF

Dear Prof Kalra

Study title:	**Pilot studies to develop and evaluate a muscle strengthening programme to reduce the risk of aspiration and improve outcome in dysphagic stroke patients.**
REC reference:	**10/H0803/32**
Protocol number:	**Protocol RMT v1.0**
Amendment number:	**Amendment 1**
Amendment date:	**21 June 2011**

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The Committee found no ethical issues.

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Participant Information Sheet	3	21 February 2011
Protocol	3	22 February 2011
Notice of Substantial Amendment (non-CTIMPs)		21 June 2011
Covering Letter		21 June 2011

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

This Research Ethics Committee is an advisory committee to London Strategic Health Authority
The National Research Ethics Service (NRES) represents the NRES Directorate within the National Patient Safety Agency and Research Ethics Committees in England

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

10/H0803/32:	Please quote this number on all correspondence

Yours sincerely

Dr Christine Heron
Chair

E-mail: kristy.randall@imperial.nhs.uk

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *Dr Zoe Harris, King's College Hospital*
Ms Jamie Peterson

NRES Committee London - Wandsworth

Attendance at Sub-Committee of the REC meeting on 15 July 2011

Name	Profession	Capacity
Professor George Hall	Professor of Anesthesia	Expert
Dr Christine Heron	Consultant Radiologist	Expert

NHS
Health Research Authority
NRES Committee London - Wandsworth
HRA Head Offices
Skipton House
80 London Road
London SE1 6LH

Tel: 020 7972 2552
Fax:

26 July 2012

Prof Lalit Kalra
Professor of Stroke Medicine
King's College London
Dept of Stroke Medicine
Academic Neurosciences Centre
King's College London
SE5 8AF

Dear Prof Kalra

Study title:	**Pilot studies to develop and evaluate a muscle strengthening programme to reduce the risk of aspiration and improve outcome in dysphagic stroke patients.**
REC reference:	**10/H0803/32**
Protocol number:	**Protocol RMT v1.0**
Amendment number:	
Amendment date:	**08 July 2012**

The above amendment was reviewed at the meeting of the Sub-Committee held on 19 July 2012 by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Participant Information Sheet: Revised PIS and consent form	4.0	05 July 2012
Protocol	4.0	05 July 2012
Notice of Substantial Amendment (non-CTIMPs)		08 July 2012
Covering Letter	From tino Kulnik	07 July 2012

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

10/H0803/32:	**Please quote this number on all correspondence**

Yours sincerely

pp

Dr Catherine Urch
Chair

E-mail: NRESCommittee.London-WestLondon@nhs.net

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *Ms Jamie Peterson*
Dr Zoe Harris, King's College Hospital

NRES Committee London - Wandsworth

Attendance at Sub-Committee of the REC meeting on 19 July 2012

Name	*Profession*	*Capacity*
Miss Maryam Alfa-Wali	Clinical Research Fellow, Academic Surgery Unit	Expert
Dr Catherine Urch	Lead Consultant in Palliative Medicine & Honorary Senior Lecturer Imperial College	None

Stefan Kulnik
Room A2.09
Academic Neuroscience Centre
Institute of Psychiatry
King's College London
Denmark Hill
London SE5 8AF

20 May 2013

Dear Stefan,

PNM/12/13-143 Study comparing the gold-standard method of peak cough flow measurement with four alternative measurement instruments

Review Outcome: Full Approval

Thank you for submitting your application for ethical approval. This was reviewed by the PNM RESC on 14 May 2013. As a result, full approval has been granted with the following provisos:

1. Sections 2.2 and 2.3: Please note that ethical approval for PhD studies is normally granted for a period of 3 years.
2. Section 7.1: The Committee recommends that participants are allowed at least 24 hours to consider whether to take part after reading the Information Sheet.
3. Section 7.2: Specify a date as the deadline for withdrawal of participant data. This should appear on the Information Sheet and Consent Form.
4. Information Sheet: State that the study has been reviewed by the Psychiatry, Nursing and Midwifery (PNM) Research Ethics Committee (RESC) at King's College London.

Please ensure that you follow all relevant guidance as laid out in the King's College London Guidelines on Good Practice in Academic Research (http://www.kcl.ac.uk/college/policyzone/index.php?id=247).

For your information ethical approval is granted until 14 May 2016. If you need approval beyond this point you will need to apply for an extension to approval at least two weeks prior to this explaining why the extension is needed, (please note however that a full re-application will not be necessary unless the protocol has changed). You should also note that if your approval is for one year, you will not be sent a reminder when it is due to lapse.

Ethical approval is required to cover the duration of the research study, up to the conclusion of the research. The conclusion of the research is defined as the final date or event detailed in the study description section of your approved application form (usually the end of data collection when all work with human participants will have been completed), not the completion of data analysis or publication of the results. For projects that only involve the further analysis of pre-existing data, approval must cover any period during which the researcher will be accessing or evaluating individual sensitive and/or un-anonymised records. Note that after the point at which ethical approval for your study is no longer

required due to the study being complete (as per the above definitions), you will still need to ensure all research data/records management and storage procedures agreed to as part of your application are adhered to and carried out accordingly.

If you do not start the project within three months of this letter please contact the Research Ethics Office.

Should you wish to make a modification to the project or request an extension to approval you will need approval for this and should follow the guidance relating to modifying approved applications: http://www.kcl.ac.uk/innovation/research/support/ethics/applications/modifications.aspx
The circumstances where modification requests are required include the addition/removal of participant groups, additions/removal/changes to research methods, asking for additional data from participants, extensions to the ethical approval period. Any proposed modifications should only be carried out once full approval for the modification request has been granted.

Any unforeseen ethical problems arising during the course of the project should be reported to the approving committee/panel. In the event of an untoward event or an adverse reaction a full report must be made to the Chair of the approving committee/review panel within one week of the incident.

Please would you also note that we may, for the purposes of audit, contact you from time to time to ascertain the status of your research.

If you have any query about any aspect of this ethical approval, please contact your panel/committee administrator in the first instance (http://www.kcl.ac.uk/innovation/research/support/ethics/contact.aspx). We wish you every success with this work.

Yours sincerely,

James Patterson - Senior Research Ethics Officer
For and on behalf of
Professor Gareth Barker, Chair
Psychiatry, Nursing and Midwifery Research Ethics Subcommittee (PNM RESC)

Cc: Lalit Kalra

Appendix 7

Participant information sheets and informed consent forms

King's College Hospital NHS
NHS Foundation Trust

PARTICIPANT INFORMATION AND CONSENT FORM

EVALUATION OF RESPIRATORY MUSCLE STRENGTHENING TO REDUCE CHEST INFECTIONS IN STROKE PATIENTS WITH SWALLOWING PROBLEMS

Many stroke patients suffer from chest infections because of swallowing problems, which result in more illness or longer hospital stay. It may be possible to reduce the number of chest infections by strengthening muscles that help in breathing and coughing in patients with swallowing problems. We are undertaking a study to see if breathing exercises can be used to strengthen these muscles and invite you to help us with this research. Please take time to read the following information carefully and discuss it with relatives and friends if you wish, before you decide whether or not you want to take part. Ask the research team if there is anything that is not clear or if you would like more information.

What is the research about?

Swallowing problems occur in nearly half of patients with stroke and are associated with an increased risk of developing pneumonia because of food, water or saliva entering the windpipe. Research shows that nearly 1 in 5 stroke patients with swallowing problems will develop pneumonia but there are very few methods available to reduce this from happening. In healthy people, cough is important for clearing airways from accidental entry of food, liquids or saliva into the windpipe. However, most stroke patients with swallowing problems have a weak cough, which is not strong enough to clear airways. There is early research which shows that relatively simple breathing exercises can be used to strengthen cough and thus improve airway clearance in patients with Parkinson's disease, chronic bronchitis and those undergoing heart surgery. However, breathing exercises to strengthen breathing muscles have not been used in stroke patients previously, despite their potential to reduce chest infections and the poor health associated with these infections.

Should I take part?

We have invited you to participate in this study because you have problems with swallowing following the stroke and are at a higher risk of food or water going down the windpipe. We believe that if proven, the exercises being tested will help people with similar problems in avoiding chest infections. The study itself will involve assessments of swallowing, breathing and cough as described below, performed before and after an exercise programme to train breathing and cough muscles. These exercises will be performed 2-5 times per day for 5 days a week for 4 weeks under the supervision of a trained research therapist. The therapist will ensure that you are able to perform these exercises without too much difficulty, fatigue or harm to yourself. It is up to you to decide whether or not to take part. Even if you decide to take part you are still free to withdraw at any time and without giving a reason. Not taking part or withdrawing will not affect

your treatment. If you withdraw, we will seek your permission to use the information already collected as a part of the study for analysis. This use will be for research purposes only and will not be traceable back to you.

What will happen to me if I take part?

If you agree to take part in the study we will:

1) Your ability to swallow will be assessed by a speech and language therapist. This test will consist of drinking and eating small quantities of food and drink of different thicknesses. This test is routinely performed on many patients even though they are not participation in research. We will measure the strength of breathing and cough muscles by asking you to blow through some equipment to measure pressures and flow rates. You will be fully supervised during the assessment. We will perform these assessments at the start and the end of the training programme. We will assess the strength of breathing and cough muscles again after 3 months.

2) Following these assessments, you will be allocated to one of the three groups; one which will be trained to strengthen muscles that help to breathe in (inspiratory muscles); the second which will be trained to strengthen muscles that help to breathe out (expiratory muscles); and the third which consist of breathing exercises for both muscle groups. The group you will be allocated to will be determined by chance; neither you nor we have any control over this decision. Muscle training will be individualised for each patient.

The training will essentially consist of teaching you either to take in a forceful deep breath against a small pressure (group 1) or to breathe out against a small pressure (group 2) using a whistle like device. The pressure against which you breathe in and out will be set at a level which is comfortable for you. Participants in group 3 will be given breathing exercises similar to groups 1 and 2 but not against any pressure. You will be asked to perform 10 such breaths (1 set). You can do as few as 2 sets and as many as 5 sets each day for 7 days a week. The whole training will continue for 4 weeks and the pressure will be reset according to your abilities at the beginning of each week. The training will be supervised by a therapist who will teach you proper device handling and provide written and verbal instructions. The therapist will see you at the beginning of each week to reset the pressure depending upon your progress and deal with any issues that you may be concerned about.

3) We will ask you to complete a daily dairy telling us how many breaths you have performed in each set and the number of sets completed over the 4 weeks. You will also be asked to write down about any problems experienced by you in the diary. The therapist will record the pressure set for the week and any other problems that you may have reported or they may have identified during the week. The training will be stopped if any significant problems with the training are identified by you or the therapist.

What are the possible risks of taking part?

There are minimal risks associated with the training. Some participants may experience transient headaches, chest pain or a small rise in blood pressure when breathing out against pressure. We will record these symptoms and measure pre- and post training blood pressure and heart rate. Training will be discontinued if complaints of headache, chest pain persist or if there is a persistent rise in blood pressure or heart rate. Some participants may complain of fatigue or inability to sustain breathing against resistance. These will be monitored and either pressures lowered or patients withdrawn from the study.

What information will be kept and will it be confidential?

In addition to the information collected on you during the study, we will collect general personal information (for example your address, contact details, date of birth, family member who can be contacted, general practitioner) and some health information (for example your medical history, health condition and activities of daily living). With your agreement, your participation in the study will be notified to your GP or other consultants who may be treating you. They may be asked to provide information on your health that is relevant to your participation in the study.

This information will only be collected by staff trained in good clinical practice for research and authorised to collect such information. The information will be anonymised so that it cannot be traced back to you and kept on a password protected computer or in secure files. All information will be used and reported in a way that none of the details can be seen by others or traced back to you.

How will I know the results of my participation?

The results of participation will not be known until we have completed the whole study and analysed the data. When we have the results, we will inform all participants of the overall findings of our research. It may not be possible to give individuals their own results because all data will be anonymised. Results from this study will also be published in medical journals and used in scientific reports.

What if something goes wrong?

If you have any cause to complain about any aspect of the study, including the way in which you have been approached or treated, please contact Prof Lalit Kalra in the first instance. If this is not satisfactory, the normal National Health Service complaints procedures apply. The Principal Investigator will be able to give you details of the hospital complaints procedure and the people to contact. All such complaints follow a rigid procedure and time-line for responding to your concerns. In the highly unlikely event that any harm may be caused by the negligence of those performing the study, the NHS indemnity guidelines will apply.

Who is overseeing the research?

This study is being organised and run by the Department of Stroke Medicine and falls within the Research Governance arrangements of the King's College Hospital NHS Foundation Trust, The Stroke Research Network and The Comprehensive Local Research Networks. The study has been reviewed by the Wandsworth Research Ethics Committee, South London REC Office, for the National Research Ethics Service. (REC No.10/H0803/32)

Contact for further information:

If you have any other questions or any concerns about the study or the way it has been carried out, you should contact any of the following:

Stefan Tino Kulnik
Research Coordinator
Tel: 020 3299 7784
Fax: 020 3299 5864

Prof Lalit Kalra
Principal Investigator
Tel: 020 3299 1718
Fax: 020 3299 3195

Dept of Clinical Neurosciences
Academic Neuroscience Centre
King's College London
King's College Hospital London SE5 8AF

If you decide you would like to take part, please read and sign the consent form. You will be given a copy of this information sheet and the signed consent form to keep.

Thank you for taking the time to read this information sheet

King's College Hospital NHS
NHS Foundation Trust

INFORMED CONSENT FORM

STUDY TITLE: EVALUATION OF RESPIRATORY MUSCLE STRENGTHENING TO REDUCE CHEST INFECTIONS IN STROKE PATIENTS WITH SWALLOWING PROBLEMS

Patient Initial: **DOB:** **Patient ID:**

Centre:

PLEASE INITIAL EACH BOX

1) I confirm that I have read and understand the Information Sheet dated 05/07/2012 for the above study. I have been given time and the opportunity to ask questions. ☐

2) I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3) I agree to take part in the above study. ☐

Name of Participant Date Signature

Name of person taking consent Date Signature

Name of Investigator (if different from above) Date Signature

1 signed original for the participant to keep, 1 signed original for site investigator file, 1 copy for notes, 1 copy to be sent to trial office

INFORMATION SHEET FOR PARTICIPANTS

REC Reference Number: PNM/12/13-143

YOU WILL BE GIVEN A COPY OF THIS INFORMATION SHEET

Study title: Comparison of cough measurement devices

We would like to invite you to participate in this postgraduate research project. You should only participate if you want to; choosing not to take part will not disadvantage you in any way. Before you decide whether you want to take part, it is important for you to understand why the research is being done and what your participation will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information.

Why we are doing this research

In this study, we compare different devices for cough testing. Measuring cough intensity (peak cough flow rate) can give useful information in clinical practice and research. Different measurement devices are in use, from elaborate laboratory-based systems to low-tech hand-held devices. These devices may vary in accuracy of measurement. In order to interpret measurements appropriately, clinicians and researchers need to know the measurement accuracy of the cough testing devices they use.
In this study, we will take cough measurements (peak cough flow rate) with four practical hand-held devices. We will then compare these with measurements made through a very accurate laboratory-based system. From this, we will be able to make recommendations for clinicians and researchers who use cough testing equipment.

Who we are looking for

We are looking for adults over the age of 18, in good general health, and willing to cough 80 times through different testing equipment.

Exclusion criteria – You will not be able to take part if you have any of the following:

- **Current or past** medical conditions of the lungs: asthma, COPD, bronchiectasis, chronic cough, cystic fibrosis, tuberculosis, pneumothorax, lung fibrosis, lung cancer, lung surgery
- **Current** cold, flu, chest infection (pneumonia), or hoarse voice
- **Current or past** medical condition of the heart: heart failure, myocardial infarction (heart attack), coronary artery narrowing, heart surgery, irregular heartbeat
- **Current or past** medical condition of the vocal cords or voice box (larynx), vocal cord palsy, surgery of the vocal cords or voice box
- History of syncopes, epilepsy, or unexplained passing out/drop attack
- Regular headaches, migraines
- Discomfort when coughing
- Pregnancy

We are looking for people from different age groups. We are also aiming for a balance of male and female participants. Therefore you may not be able to take part, depending on who has taken part already.

If you agree to take part

If you are interested in the study, please email the researcher (stefan.kulnik@kcl.ac.uk). This information sheet will then be emailed to you. With your agreement, the researcher will speak to you by telephone to answer any questions you may have and talk you through the list of exclusion criteria (see above).

If you agree to take part, the researcher will make an appointment with you to attend one single testing session at your convenience. The session will take approximately 75 minutes. We are happy to arrange any time that suits you from Monday to Friday, between 7am and 8pm, and on Saturdays between 9am and 2pm. It will take place at the Denmark Hill Campus of King's College London.

At the beginning of the session, you will be asked to complete and sign the written consent form. The researcher will take a note of your age. You will then be asked to cough 20 times through four different cough testing devices. In all, you will be asked to give a total of 40 maximally strong coughs, 20 'medium' coughs, and 20 'mini-coughs'. You will take rests between coughs and while equipment is changed over. Drinking water and cough lozenges will be provided to soothe your throat as required.

After the testing session, we will store the measurement results electronically. From this point on the results will be anonymised, which means that it will no longer be possible to link the results to your person. We will then use statistical computer software to analyse the level of accuracy between the different cough testing devices.

Are there any risks when taking part?

The researcher will follow the same strict hygiene procedures as clinical lung function laboratories. You will be protected by a bacterial filter, which is fitted to the testing devices and creates a barrier between you and the equipment. The risk of infection from the equipment is negligible.

Although we do not expect it, it may be that coughing repeatedly causes you strain or discomfort of the throat. To minimise this risk, you will take rests between coughs. Drinking water and cough lozenges are provided as required. Should you find the testing session too uncomfortable, you are free to stop and quit at any time.

Are there any benefits to taking part?

We do not anticipate that taking part will bring any benefits to your health. Unfortunately, we are not able to compensate you for your time or travelling costs.
After the study is completed, we will produce a short summary of the findings, which we can email to you for your information.

0

Anonymity and confidentiality

All information collected for this study will be handled in accordance with the terms of the UK Data Protection Act 1998. You will give your name and email address when completing the written consent form. According to the college's regulations, these forms will be kept secure in a locked filing cabinet at the researcher's office for the duration of five years. The forms will then be destroyed. The researcher will also take note of your age and your cough measurements. This information will be anonymised, which means that it cannot be traced back to you in any articles, presentations, or other reporting of this study.

What we will do with the study findings

We will use the findings to test the accuracy, validity and comparability of portable cough testing devices commonly used in research and clinics. This will improve the quality of our research and be useful for other clinicians and researchers who do cough measurements. We will disseminate these findings using presentations at professional societies and in scientific journals. The study findings will also be summarised in the researcher's PhD thesis.

It is up to you to decide whether to take part in this study or not. If you decide to take part you are still free to withdraw from the study at any time and without giving a reason. Your data can be withdrawn from the study until it has been processed electronically for analysis, which is until the 30th November 2013.

If you have any questions or require more information about this study, please contact the researcher using the following contact details:

Stefan Tino Kulnik, Research Worker
Academic Neuroscience Centre, ANC-Building, PO Box 41
Institute of Psychiatry, King's College London
Denmark Hill, London SE5 8AF
Tel.: 020 3299 7784
Email: stefan.kulnik@kcl.ac.uk

If this study has harmed you in any way, you can contact King's College London using the details below for further advice and information:

Prof Lalit Kalra
Department of Clinical Neuroscience
Academic Neuroscience Centre, ANC-Building, PO Box 41
Institute of Psychiatry, King's College London
Denmark Hill, London SE5 8AF
Tel.: 020 3299 1718
Email: lalit.kalra@kcl.ac.uk

CONSENT FORM FOR PARTICIPANTS IN RESEARCH STUDIES

Please complete this form after you have read the Information Sheet and/or listened to an explanation about the research.

Title of Study: Comparison of cough measurement devices

King's College Research Ethics Committee Ref: PNM/12/13-143

Thank you for considering taking part in this research. The person organising the research must explain the project to you before you agree to take part. If you have any questions arising from the Information Sheet or explanation already given to you, please ask the researcher before you decide whether to join in. You will be given a copy of this Consent Form to keep and refer to at any time.

Please tick or initial

- **I understand that if I decide at any time during the research that I no longer wish to participate in this project, I can notify the researchers involved and withdraw from it immediately without giving any reason. Furthermore, I understand that I will be able to withdraw my data until it is processed electronically for analysis (until 30th November 2013).** ☐
- **I consent to the processing of my personal information for the purposes explained to me. I understand that such information will be handled in accordance with the terms of the UK Data Protection Act 1998.** ☐
- **I understand that I must not take part if any of the following apply to me, and I confirm that none of the following apply to me:** ☐
  - **Current or past** medical conditions of the lungs: asthma, COPD, bronchiectasis, chronic cough, cystic fibrosis, tuberculosis, pneumothorax, lung fibrosis, lung cancer, lung surgery
  - **Current** cold, flu, chest infection (pneumonia), or hoarse voice
  - **Current or past** medical condition of the heart: heart failure, myocardial infarction (heart attack), coronary artery narrowing, heart surgery, irregular heartbeat
  - **Current or past** medical condition of the vocal cords or voice box (larynx), vocal cord palsy, surgery of the vocal cords or voice box
  - History of syncopes, epilepsy, or unexplained passing out/drop attack
  - Regular headaches, migraines
  - Discomfort when coughing
  - Pregnancy

- I would like to receive a summary of the study findings by email. (If yes, please provide email address:)

Yes	No

Participant's Statement:

I _____

agree that the research project named above has been explained to me to my satisfaction and I agree to take part in the study. I have read both the notes written above and the Information Sheet about the project, and understand what the research study involves.

Signed **Date**

Investigator's Statement:

I _____

confirm that I have carefully explained the nature, demands and any foreseeable risks (where applicable) of the proposed research to the participant.

Signed **Date**

Appendix 8

Data collection forms

Stroke - Respiratory Muscle Training Study

A randomised controlled trial to determine the feasibility and effects of inspiratory and/or expiratory muscle training in acute stroke patients with dysphagia

Case Report Form

Instructions for use of CRF form:

1. Please Complete all entries using pen
2. All dates should be entered in the dd/mm/yyyy format; times should be entered in the 24hr clock hhmm format
3. When completed ensure page 3 is returned to the trial coordinator and separated from the master file
4. Please ensure the complete form is returned to the trial coordinator and placed in the master file
5. In the case of any adverse event ensure the adverse event (page 11) are completed and returned to the trial coordinator ASAP

Contacts:

Stefan Tino Kulnik
Research Worker
Academic Neuroscience Centre
PO Box 41, Institute of Psychiatry
Denmark Hill
London SE5 8AF

Tel: 0203 299 7784
Fax: 0203 299 5864
e-mail: stefan.kulnik@kcl.ac.uk

Professor Lalit Kalra
Chief Investigator
Academic Neuroscience Centre
PO Box 41, Institute of Psychiatry
Denmark Hill
London SE5 8AF

Tel: 0203 299 3487
Fax: 0203 014 8891
e-mail: lalit.kalra@kcl.ac.uk

Co-Sponsors:

Research and Development Department
King's College Hospital NHS Foundation Trust
Jennie Lee House, Love Walk
Denmark Hill
London SE5 8AD

Patient Initial: **DOB:** **Patient ID:**

Screening:

Date of stroke onset: Date of Admission:

Checklist of Inclusion:

1) Stroke	Yes ☐	No ☐
2) Aged greater than 18	Yes ☐	No ☐
3) Within 2 weeks of stroke	Yes ☐	No ☐
4) Score of ≥ 5 on NIH stroke scale	Yes ☐	No ☐

Checklist of Exclusion:

1) Is BP ≥180/100 mmHg (at least 3x in past 24 hours)	Yes ☐	No ☐
2) In the previous 3 months has the patient had		
a) Myocardial infarction	Yes ☐	No ☐
b) Angina	Yes ☐	No ☐
c) Heart failure	Yes ☐	No ☐
3) Has the patient exhibited features of raised intracortical pressure on CT scan	Yes ☐	No ☐
6) Does the patient have any pulmonary, neurological (other than stroke) or orthopaedic conditions that may affect the respiratory pump	Yes ☐	No ☐

Consent Procedures:

Informed consent obtained: Yes [ ] No [ ]

Obtained by: Date: Time:

Date of inclusion into the study:

Patient Initial: **DOB:** **Patient ID:**

Patient Information

What age is the patient (years):

What height is the patient (meters):

What Weight is the patient (kg):

What sex is the patient:

What was the date of the patients stroke:

Does the patient smoke:

Stroke Side:

NIH score:

What group has the patient been allocated to:

Patient Initial: **DOB:** **Patient ID:**

Nottingham Extended ADL Scale

	Baseline	4 weeks	12 weeks
Date:			
1. Walk around outside?			
Not at all			
With help			
On your ow n w ith difficulty			
On your ow n			
2. Climb stairs?			
Not at all			
With help			
On your ow n w ith difficulty			
On your ow n			
3. Getting in and out of a car?			
Not at all			
With help			
On your ow n w ith difficulty			
On your ow n			
4. Walk over uneven ground?			
Not at all			
With help			
On your ow n w ith difficulty			
On your ow n			
5. Cross roads?			
Not at all			
With help			
On your ow n w ith difficulty			
On your ow n			
6. Travel of public transport?			
Not at all			
With help			
On your ow n w ith difficulty			
On your ow n			
7. Manage to feed yourself?			
Not at all			
With help			
On your ow n w ith difficulty			
On your ow n			
8. Manage to make yourself a hot drink?			
Not at all			
With help			
On your ow n w ith difficulty			
On your ow n			
9. Take hot drinks from one room to another?			
Not at all			
With help			
On your ow n w ith difficulty			
On your ow n			
10. Do the w ashing up?			
Not at all			
With help			
On your ow n w ith difficulty			
On your ow n			
11. Make yourself a hot snack?			
Not at all			
With help			
On your ow n w ith difficulty			
On your ow n			

Signature: Date: 4

Patient Initial: **DOB:** **Patient ID:**

Nottingham Extended ADL Scale Cont.

	Baseline	4 weeks	12 weeks
Date:			
12. Manage your own money when out?			
Not at all			
With help			
On your own with difficulty			
On your own			
13. Wash small items of clothing?			
Not at all			
With help			
On your own with difficulty			
On your own			
14. Do your own housework?			
Not at all			
With help			
On your own with difficulty			
On your own			
15. Do your own shopping?			
Not at all			
With help			
On your own with difficulty			
On your own			
16. Do a full clothes wash?			
Not at all			
With help			
On your own with difficulty			
On your own			
17. Read newspapers or books?			
Not at all			
With help			
On your own with difficulty			
On your own			
18. Use the telephone?			
Not at all			
With help			
On your own with difficulty			
On your own			
19. Write letters?			
Not at all			
With help			
On your own with difficulty			
On your own			
20. Go out socially?			
Not at all			
With help			
On your own with difficulty			
On your own			
21. Manage your own garden?			
Not at all			
With help			
On your own with difficulty			
On your own			
22. Drive a car?			
Not at all			
With help			
On your own with difficulty			
On your own			

Signature: Date: 5

Patient Initial: **DOB:** **Patient ID:**

Respiratory Function Testing:

		Baseline	1st week	2nd week	3rd week	4th week	12th week
Date:							
FEV1 (Litres)	1						
	2						
	3						
	Best						
FVC (Litres)	1						
	2						
	3						
	Best						
PEFR (Litres/min)	1						
	2						
	3						
	Best						

PEmax (cmH2O)	1						
	2						
	3						
	4						
	5						
	6						
	7						
	8						
	9						
	10						
	Best						
	2nd Best						
	3rd Best						
	Mean						
PImax (cmH2O)	1						
	2						
	3						
	4						
	5						
	6						
	7						
	8						
	9						
	10						
	Best						
	2nd Best						
	3rd Best						
	Mean						

Patient Initial: **DOB:** **Patient ID:**

Assessment of Aspiration (Clinical examination):
Please indicate whether the patients swallow is safe or unsafe

	Baseline	4 Weeks	12 Weeks
Safe	☐	☐	☐
Unsafe	☐	☐	☐

Assessment of Cough:

Voluntary Cough: Biggest cough after maximal inspiration (performed until 3 peak flows within 10%). Best attempt recorded

		Peak Inspiratory Flow Rate (L/min)	Peak Expiratory Flow Rate (L/min)	Expired Volume (Litres)	Inspired Volume (Litres)	Cough Volume Acceleration (L/s^2)	Compression Time (s)
Baseline	1						
	2						
	3						
	4						
	5						
	Best						
4 Weeks	1						
	2						
	3						
	4						
	5						
	Best						
12 Weeks	1						
	2						
	3						
	4						
	5						
	Best						

Cough Assessment Continued on Next Page

Patient Initial: **DOB:** **Patient ID:**

Reflex Cough: induced by nebulising capsiacin. Best attempt recorded

		Peak Inspiratory Flow Rate (L/min)	Peak Expiratory Flow Rate (L/min)	Expired Volume (Litres)	Inspired Volume (Litres)	Cough Volume Acceleration (L/s^2)	Compression Time (s)
Baseline	1						
	2						
	3						
	4						
	5						
	Best						
4 Weeks	1						
	2						
	3						
	4						
	5						
	Best						
12 Weeks	1						
	2						
	3						
	4						
	5						
	Best						

Assessment of Chest Infection:

Please indicate whether the patient has any of the following:

	Baseline	4 weeks	12 weeks
Self reported chest infection	[ ] Yes [ ] No	[ ] Yes [ ] No	[ ] Yes [ ] No
Use of antibiotics for chest symptoms	[ ] Yes [ ] No	[ ] Yes [ ] No	[ ] Yes [ ] No
Hospitalisation due to chest problems	[ ] Yes [ ] No	[ ] Yes [ ] No	[ ] Yes [ ] No
If Yes to any of the above the patient is classified as having had a chest infection			
Chest Infection	[ ] Yes [ ] No	[ ] Yes [ ] No	[ ] Yes [ ] No

Signature: Date: 8

Patient Initial: **DOB:** **Patient ID:**

Safety Monitoring:

Please complete at the beginning and end of the first training session after the pressure threshold is increased

		Blood Pressure	Heart Rate	O_2 Saturation	Adverse Reactions
Baseline	Pre				
	Post				
End week 1	Pre				
	Post				
End week 2	Pre				
	Post				
End week 3	Pre				
	Post				

At any time point did the patient have any of the following:

	Baseline	End week 1	End week 2	End week 3
Systolic BP < 110 or > 180 mmHg	[ ] Yes [ ] No	[ ] Yes [ ] No	[ ] Yes [ ] No	[ ] Yes [ ] No
Diastolic BP > 105 mmHg	[ ] Yes [ ] No	[ ] Yes [ ] No	[ ] Yes [ ] No	[ ] Yes [ ] No
HR < 60 or > 120 bpm	[ ] Yes [ ] No	[ ] Yes [ ] No	[ ] Yes [ ] No	[ ] Yes [ ] No
O_2 Saturation < 90%	[ ] Yes [ ] No	[ ] Yes [ ] No	[ ] Yes [ ] No	[ ] Yes [ ] No
Complaint of:				
Headache	[ ] Yes [ ] No	[ ] Yes [ ] No	[ ] Yes [ ] No	[ ] Yes [ ] No
Chest pain	[ ] Yes [ ] No	[ ] Yes [ ] No	[ ] Yes [ ] No	[ ] Yes [ ] No
Strain	[ ] Yes [ ] No	[ ] Yes [ ] No	[ ] Yes [ ] No	[ ] Yes [ ] No

If the answer is *yes* to any of the above discontinue training immediately

If applicable please specify the date and reason for discontinuation:

Date of Discontinuation:

Reason:

Signature: Date: 9

Patient Initial: **DOB:** **Patient ID:**

ADVERSE EVENT FORM

Please document any adverse events during study participation, whether or not these are related to participation. Please use one form for each event.

Definition of a Serious Adverse Event (SAE)

An adverse event is classified as a SAE if it meets the following conditions:

- **Results in death**
- **Is life threatening***
- **Requires hospitalisation or prolongation of existing hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Requires intervention to prevent permanent impairment or damage**

**an adverse experience that the Investigator considers to have actually placed the subject at immediate risk from death, not merely had the potential to do so had it been more severe.*

Has the patient suffered from a:

Serious Adverse Event	**Yes [ ]**	**No [ ]**
Minor Adverse Event	**Yes [ ]**	**No [ ]**

Please give details:

Please report all serious expected or unexpected adverse events within 24 hours to the Chief Investigator (020 3299 1719 or 020 3299 3487) or trial coordinator (0207 848 0185) immediately

Signature: Date: 10

Stroke - Respiratory Muscle Training Study

A randomised controlled trial to determine the feasibility and effects of inspiratory and/or expiratory muscle training in acute stroke patients with dysphagia

Patient Contact Sheet

Patient Name:
Date of Birth:
Address:

Phone Number:

Next of Kin:
Address:

Phone Number:

Centre:
Contact:
Number:

General Practitioner:
Address:

Phone Number:

Patient Initials:	**DOB:**	**Patient ID:**
Centre:	**Centre Code:**	

Remove this sheet from the case report form and keep in a secure folder with access restricted to authorised research staff

Signature: Date: 11

Sub-Set Test-Retest Reliability Data

Tester:

Subject:

Date:

First round of testing

Time:

Forced spirometry

	1	2	3
FEV1			
FVC			
PEF			
Best of 3			

Maximum mouth pressures

	MEP	MIP
Attempt 1		
Attempt 2		
Attempt 3		
Attempt 4		
Attempt 5		
(Attempt 6)		
(Attempt 7)		
(Attempt 8)		
(Attempt 9)		
(Attempt 10)		

Best		
2nd best		
3rd best		
Mean		

Room T: BTPS correction factor applied for inspiratory cough parameters:

Voluntary cough

	PIFR	PEFR	VE	VI	Accel	Glottis
1						
2						
3						
4						
5						
Best						

Reflex cough

	PIFR	PEFR	VE	VI	Accel	Glottis
1						
2						
3						
4						
5						
Best						

Second round of testing

Time:

Forced spirometry

	1	2	3
FEV1			
FVC			
PEF			
Best of 3			

Maximum mouth pressures

	MEP	MIP
Attempt 1		
Attempt 2		
Attempt 3		
Attempt 4		
Attempt 5		
(Attempt 6)		
(Attempt 7)		
(Attempt 8)		
(Attempt 9)		
(Attempt 10)		
Best		
2nd best		
3rd best		
Mean		

Cough flow measurement system re-calibrated prior to second round testing ☐

Room T: BTPS correction factor applied for inspiratory cough parameters:

Voluntary cough

	PIFR	PEFR	VE	VI	Accel	Glottis
1						
2						
3						
4						
5						
Best						

Reflex cough

	PIFR	PEFR	VE	VI	Accel	Glottis
1						
2						
3						
4						
5						
Best						

Patient ID: Date of Training Commencement:

Respiratory Exercise Training Log

Training Protocol:

1. Respiratory training should be undertaken for 4 weeks
2. Training should be performed 7 times per week
3. In each training session 3 to 5 sets should be performed
4. One set consists of performing 10 breaths while breathing through the training device **(the nose clip should be worn during each set)**
5. You should rest for 1 minute between each training set

Set 1	1 min Rest	Set 2	1 min Rest	Set 3	1 min Rest	Set 4	1 min Rest	Set 5

For each set take 10 consecutive breaths while breathing through the training device

During each training session the training log (Pages 2 - 5) should be completed

1. **Record the date and time of the training session**
2. **For each set performed please write down the number of breaths taken**
3. **Please also indicate the pressure threshold of the device (see page 6)**
4. **In the final column please place any comments about training or describe any adverse reactions to the training**

example of how to fill out the training log:

Date & Time	Number of training breaths	Pressure threshold	Comments
Day 1 *Tuesday 01 March 2011, 2pm*	Set 1 - *10* Set 2 - *10* Set 3 – *8* Set 4 - *none* Set 5 - *none*	*12*	*Feeling a little tired afterwards*
Day 2 *Wednesday 02 March, 10am*	Set 1 - *10* Set 2 - *10* Set 3 – *10* Set 4 – *10* Set 5 – *10*	*12*	*Went well*

In this example on the first day 10 breaths were taken during the first 2 sets while only 8 were taken on the third set. The pressure threshold was 12.

On the second day all 5 sets were performed with 10 breaths taken in each set.

Instructions for training device assembly and use are given at the end of the booklet (Page 6)

1

Patient ID: Date of Training Commencement:

Date & Time	Number of training breaths	Pressure threshold	Comments
Day 1	Set 1 Set 2 Set 3 Set 4 Set 5		
Day 2	Set 1 Set 2 Set 3 Set 4 Set 5		
Day 3	Set 1 Set 2 Set 3 Set 4 Set 5		
Day 4	Set 1 Set 2 Set 3 Set 4 Set 5		
Day 5	Set 1 Set 2 Set 3 Set 4 Set 5		
Day 6	Set 1 Set 2 Set 3 Set 4 Set 5		
Day 7	Set 1 Set 2 Set 3 Set 4 Set 5		

Patient ID: Date of Training Commencement:

Date & Time	Number of training breaths	Pressure threshold	Comments
Day 8	Set 1 Set 2 Set 3 Set 4 Set 5		
Day 9	Set 1 Set 2 Set 3 Set 4 Set 5		
Day 10	Set 1 Set 2 Set 3 Set 4 Set 5		
Day 11	Set 1 Set 2 Set 3 Set 4 Set 5		
Day 12	Set 1 Set 2 Set 3 Set 4 Set 5		
Day 13	Set 1 Set 2 Set 3 Set 4 Set 5		
Day 14	Set 1 Set 2 Set 3 Set 4 Set 5		

Patient ID: Date of Training Commencement:

Date & Time	Number of training breaths	Pressure threshold	Comments
Day 15	Set 1 Set 2 Set 3 Set 4 Set 5		
Day 16	Set 1 Set 2 Set 3 Set 4 Set 5		
Day 17	Set 1 Set 2 Set 3 Set 4 Set 5		
Day 18	Set 1 Set 2 Set 3 Set 4 Set 5		
Day 19	Set 1 Set 2 Set 3 Set 4 Set 5		
Day 20	Set 1 Set 2 Set 3 Set 4 Set 5		
Day 21	Set 1 Set 2 Set 3 Set 4 Set 5		

Patient ID: Date of Training Commencement:

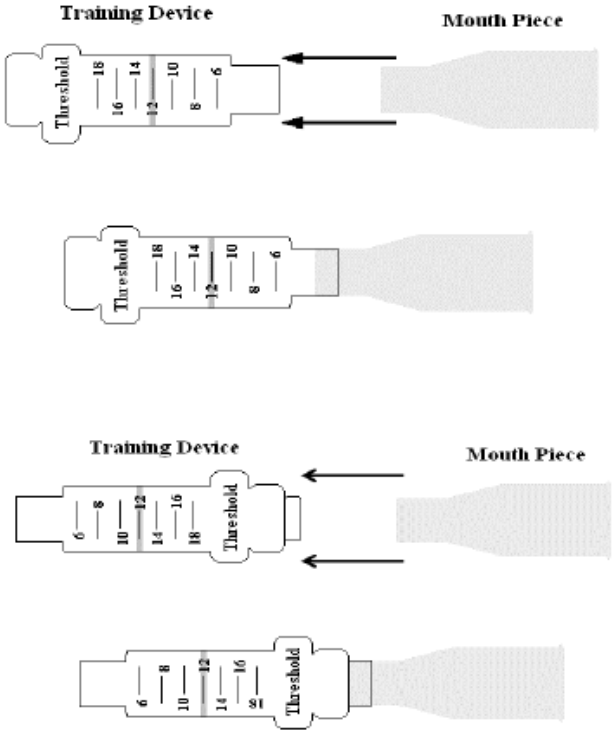
Date & Time	Number of training breaths	Pressure threshold	Comments
Day 22	Set 1 Set 2 Set 3 Set 4 Set 5		
Day 23	Set 1 Set 2 Set 3 Set 4 Set 5		
Day 24	Set 1 Set 2 Set 3 Set 4 Set 5		
Day 25	Set 1 Set 2 Set 3 Set 4 Set 5		
Day 26	Set 1 Set 2 Set 3 Set 4 Set 5		
Day 27	Set 1 Set 2 Set 3 Set 4 Set 5		
Day 28	Set 1 Set 2 Set 3 Set 4 Set 5		

5

Patient ID: Date of Training Commencement:

Training Device Set-up and Use

1. Insert the mouth piece into the training device as shown below
2. Ensure that you do not rotate the mouth piece when inserted into the device as this will alter the pressure threshold
3. Make a note of the pressure threshold the device is set at (in the example below the grey bar indicates a pressure threshold of 12. On the actual training device this will be indicated by a red bar)
4. **Put on the nose clip**
5. Place your mouth over the mouth piece forming a tight seal around it with you lips
6. Breathe as you normally would (note that when breathing in OR out you may find it more difficult than normal)



6

Case Record Form – COUGHS

Study Title: **Comparison of cough measurement devices**

Contact Person: Stefan Tino Kulnik, Stroke Research Team, Academic Neuroscience Centre, ANC-Building, PO Box 41, Institute of Psychiatry, King's College London, Denmark Hill, London SE5 8AF, Tel 020 3299 7784, stefan.kulnik@nhs.net

Participant number: _____ Date of testing: _____

Participant age: _____ Room temperature: _____

Condition of testing see protocol Table 1	Peak Flow (litres/minute)									
	Alternative device	Pneumo-tachograph	Alternative device	Pneumo-tachograph	Alternative device	Pneumo-tachograph	Alternative device	Pneumo-tachograph	Alternative device	Pneumo-tachograph
Device										
Device										
Device										
Device										

Name: _____ Date: _____ Signed: _____

Table 1. Order of testing, determined randomly. 1, 2, 3, 4 indicate which device is tested. A, B indicate whether the subject first coughs through the device in series with the pneumotachograph or through the device alone.

Subject	First device tested	Second device tested	Third device tested	Fourth device tested
1	4-B	2-A	1-B	3-B
2	1-A	2-B	4-A	3-B
3	4-A	2-A	3-B	1-A
4	3-A	4-A	1-A	2-B
5	3-B	4-B	1-A	2-B
6	3-B	4-B	2-B	1-B
7	4-B	1-B	3-B	2-B
8	2-A	4-B	1-B	3-B
9	1-B	4-A	2-B	3-B
10	1-A	4-A	3-B	2-B
11	2-B	3-A	4-B	1-A
12	2-A	3-A	1-A	4-A
13	2-B	1-B	3-A	4-B
14	4-B	3-A	1-A	2-B
15	3-B	2-B	1-B	4-B
16	3-A	1-A	2-A	4-A
17	3-A	2-A	1-B	4-A
18	2-B	1-B	3-B	4-A
19	3-A	4-A	1-B	2-A
20	3-B	2-B	4-A	1-B

1, CareFusion SprioUSB hand-held spirometer
2, Mini-Wright Peak Flow Meter Standard EU Scale
3, Assess Peak Flow Meter Standard Range 710
4, CareFusion Microlab hand-held spirometer

A, pneumotachograph and alternative device in series first, then alternative device alone
B, alternative device alone first, then in series with pneumotachograph

Case Record Form – FORCED EXPIRATORY MANOEVRES

Study Title: **Comparison of cough measurement devices**

Contact Person: Stefan Tino Kulnik, Stroke Research Team, Academic Neuroscience Centre, ANC-Building, PO Box 41, Institute of Psychiatry, King's College London, Denmark Hill, London SE5 8AF, Tel 020 3299 7784, stefan.kulnik@nhs.net

Participant number: _____ Date of testing: _____

Participant age: _____ Room temperature: _____

Condition of testing see protocol Table 1	Peak Flow (litres/minute)									
	Alternative device	Pneumo-tachograph	Alternative device	Pneumo-tachograph	Alternative device	Pneumo-tachograph	Alternative device	Pneumo-tachograph	Alternative device	Pneumo-tachograph
Device										
Device										
Device										
Device										

Name: _____ Date: _____ Signed: _____

Table 1. Order of testing, determined randomly. 1, 2, 3, 4 indicate which device is tested. A, B indicate the sequence of maximal and submaximal efforts on forced expiratory manoeuvres.

Subject	First device tested	Second device tested	Third device tested	Fourth device tested
1	4-B	2-A	1-B	3-B
2	1-A	2-B	4-A	3-B
3	4-A	2-A	3-B	1-A
4	3-A	4-A	1-A	2-B
5	3-B	4-B	1-A	2-B
6	3-B	4-B	2-B	1-B
7	4-B	1-B	3-B	2-B
8	2-A	4-B	1-B	3-B
9	1-B	4-A	2-B	3-B
10	1-A	4-A	3-B	2-B
11	2-B	3-A	4-B	1-A
12	2-A	3-A	1-A	4-A
13	2-B	1-B	3-A	4-B
14	4-B	3-A	1-A	2-B
15	3-B	2-B	1-B	4-B
16	3-A	1-A	2-A	4-A
17	3-A	2-A	1-B	4-A
18	2-B	1-B	3-B	4-A
19	3-A	4-A	1-B	2-A
20	3-B	2-B	4-A	1-B

1, CareFusion SprioUSB hand-held spirometer
2, Mini-Wright Peak Flow Meter Standard EU Scale
3, Assess Peak Flow Meter Standard Range 710
4, CareFusion Microlab hand-held spirometer

A, four alternating sets of five forced expiratory manoeuvres, starting with maximal effort, then submaximal effort
B, four alternating sets of five forced expiratory manoeuvres, starting with submaximal effort, then maximal effort

Case Record Form Study Title: **Comparison of cough measurement devices**

Contact Person: Stefan Tino Kulnik, Stroke Research Team, Academic Neuroscience Centre, ANC-Building, PO Box 41, Institute of Psychiatry, King's College London, Denmark Hill, London SE5 8AF, Tel 020 3299 7784, stefan.kulnik@nhs.net

Laboratory testing of the 4 alternative measurement devices, using a mechanically simulated cough (pressurised barrel and balloon valve)

PNT = pneumotachograph
Mini-Wright = Mini-Wright Peak Flow Meter
Assess = Assess Peak Flow Meter
SpiroUSB = CareFusion SpiroUSB hand-held spirometer
Microlab = CareFusion Microlab hand-held spirometer

Round ___: Target flow approx _____ litres/minute

Baseline PNT measurements – to demonstrate consistency of the mechanically generated "cough" flow wave:

	PNT #1	PNT #2	PNT #3	PNT #4	PNT #5
Valve opened at this pressure level (mmH2O)					
Peak flow measured (L/Min)					
Volume released (L, as per PNT measurement)					
Rise time from zero flow to peak flow (sec)					

Mini-Wright:

DEVICE ALONE	Mini-Wright #1	Mini-Wright #2	Mini-Wright #3	Mini-Wright #4	Mini-Wright #5	PNT (check 'cough' is constant)	
Valve opened at this pressure level (mmH2O)						Valve opened at this pressure level (mmH2O)	
Peak flow measured by Mini-Wright (L/Min)						Peak flow measured by PNT (L/Min)	
						Volume released (L, as per PNT measurement)	
						Rise time from zero flow to peak flow (sec)	
DEVICE IN SERIES WITH PNT	Mini-Wright #1	Mini-Wright #2	Mini-Wright #3	Mini-Wright #4	Mini-Wright #5	PNT (check 'cough' is constant)	
Valve opened at this pressure level (mmH2O)						Valve opened at this pressure level (mmH2O)	
Peak flow measured by Mini-Wright (L/Min)						Peak flow measured by PNT (L/Min)	
Peak flow measured by PNT (L/Min						Volume released (L, as per PNT measurement)	
						Rise time from zero flow to peak flow (sec)	

Assess:

DEVICE ALONE	Assess #1	Assess #2	Assess #3	Assess #4	Assess# 5	PNT (check 'cough' is constant)	
Valve opened at this pressure level (mmH2O)						Valve opened at this pressure level (mmH2O)	
Peak flow measured by Assess (L/Min)						Peak flow measured by PNT (L/Min)	
						Volume released (L, as per PNT measurement)	
						Rise time from zero flow to peak flow (sec)	
DEVICE IN SERIES WITH PNT	Assess #1	Assess #2	Assess #3	Assess #4	Assess #5	PNT (check 'cough' is constant)	
Valve opened at this pressure level (mmH2O)						Valve opened at this pressure level (mmH2O)	
Peak flow measured by Assess (L/Min)						Peak flow measured by PNT (L/Min)	
Peak flow measured by PNT (L/Min						Volume released (L, as per PNT measurement)	
						Rise time from zero flow to peak flow (sec)	

SpiroUSB:

DEVICE ALONE	SpiroUSB #1	SpiroUSB #2	SpiroUSB #3	SpiroUSB #4	SpiroUSB #5	PNT (check 'cough' is constant)	
Valve opened at this pressure level (mmH2O)						Valve opened at this pressure level (mmH2O)	
Peak flow measured by SpiroUSB (L/Min)						Peak flow measured by PNT (L/Min)	
						Volume released (L, as per PNT measurement)	
						Rise time from zero flow to peak flow (sec)	
DEVICE IN SERIES WITH PNT	SpiroUSB #1	SpiroUSB #2	SpiroUSB #3	SpiroUSB #4	SpiroUSB #5	PNT (check 'cough' is constant)	
Valve opened at this pressure level (mmH2O)						Valve opened at this pressure level (mmH2O)	
Peak flow measured by SpiroUSB (L/Min)						Peak flow measured by PNT (L/Min)	
Peak flow measured by PNT (L/Min						Volume released (L, as per PNT measurement)	
						Rise time from zero flow to peak flow (sec)	

Microlab:

DEVICE ALONE	Microlab #1	Microlab #2	Microlab #3	Microlab #4	Microlab #5	PNT (check 'cough' is constant)	
Valve opened at this pressure level (mmH2O)						Valve opened at this pressure level (mmH2O)	
Peak flow measured by Microlab (L/Min)						Peak flow measured by PNT (L/Min)	
						Volume released (L, as per PNT measurement)	
						Rise time from zero flow to peak flow (sec)	
DEVICE IN SERIES WITH PNT	Microlab #1	Microlab #2	Microlab #3	Microlab #4	Microlab #5	PNT (check 'cough' is constant)	
Valve opened at this pressure level (mmH2O)						Valve opened at this pressure level (mmH2O)	
Peak flow measured by Microlab (L/Min)						Peak flow measured by PNT (L/Min)	
Peak flow measured by PNT (L/Min						Volume released (L, as per PNT measurement)	
						Rise time from zero flow to peak flow (sec)	